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Parkinson's disease (PD) is characterized by a loss of substantia nigra dopaminergic neurons. Here we describe our progress in understanding the role of metabotropic glutamate receptors (mGluRs) as a novel target for the treatment of PD. We have localized mGluR4 in basal ganglia structures, and explored its role in mediating the electrophysiological effects of glutamate in rat brain slices. We have explored the efficacy of mGluR drugs in relieving motor symptoms in hemi-parkinsonian monkeys. We found that group III mGluRs are presynaptic on striatal-pallidal terminals and that they mediate a reduction in IPSC amplitude in the SNr. They also pre-synaptically inhibit EPSCs at the STN-SNr synapse. In this study, we have found that Groups I and II mGluRs also have critical roles in regulating basal ganglia function. Group II mGluRs mediate a presynaptic reduction of EPSCs in the SNr and group II agonist LY354740 reverses catalepsy in (a rodent) model of PD. Post-synaptic Group I mGluRs are involved in regulation of BG output nuclei by both excitation and disinhibition. Furthermore, comprehensive characterization of the roles of mGluRs in the basal ganglia raises the possibility that they may provide targets for novel therapeutic agents for treatment of PD.

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INTRODUCTON

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by disabling motor impairments including tremor, rigidity, and bradykinesia. In PD there is a significant loss of nigrostriatal dopamine neurons that results in a series of neurophysiological changes that lead to a pathological excitation of the subthalamic nucleus (STN). The increased activity of STN neurons leads to an increase in glutamate release at STN synapses onto GABAergic projection neurons in the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). This glutamate-mediated over excitation of the BG output nuclei ultimately leads to a "shutdown" of thalamocortical projections and produces the motor impairments characteristic of PD[1]. Unfortunately, as the disease progresses, the efficacy of traditional dopamine replacement therapy becomes severely diminished and severe motor and psychiatric side effects can occur [2] . Because of this, a great deal of effort has been focused on developing new approaches for the treatment of PD. In these studies we are pursuing a novel therapeutic approach by targeting drugs acting at metabotropic glutamate receptors (mGluRs). Eight mGluR subtypes have been cloned (designated mGluR1-mGluR8) from mammalian brain. Each subtype is classified in one of three classes (I-III). Although the present research focuses on group III mGluRs, in particular mGluR4, during the course of our investigation, we discovered that Groups I and II mGluRs also play a crucial role in regulating BG function. Consequently, we have expanded our mGluR studies to include receptors from the other two groups. To date we have developed and characterized antibodies to mGluR4 which were used to generate a map of receptor distribution in the rat basal ganglia. We determined the effects of selective group III mGluR agonists and antagonists on synaptic transmission at the major excitatory synapses in the SNr and GPi. We characterized some of the effects of agonists and antagonists to groups I and II at different BG loci. Finally, we have been evaluating the therapeutic potential of Groups I, II, and III agonists and antagonists in hemi-parkinsonian monkeys. Since mGluRs play an important role in the modulation of BG function, we expect that the results of this study may provide valuable insight into alternative treatment options for PD.

BODY

Specific Aim I.

To localize mGluR4a and 4b receptors in rat and monkey basal ganglia by immunohistochemical techniques using subtype- and isoform- specific antibodies.

This aim is focused on the development and characterization of antibodies against mGluR4. The antibodies are used as a tool to reveal the anatomical distribution of these receptors in the basal ganglia. Previously we reported on the successful development and characterization of a polyclonal antibody to the carboxy terminal portion of mGluR4a. Immunocytochemistry (ICC) was used to visualize the distribution and localization of mGluR4a in the rat brain. Very low levels of mGluR4a were detected in the striatum (STR). Virtually no staining was detected in the substantia nigra pars compacta (SNc). The substantia nigra pars reticulata (SNr), entopeduncular nucleus (EPN), and globus pallidus (GP), however exhibited densely labeled neuronal fibers. Electron and confocal microscopy was used to confirm the localization of this receptor to presynaptic terminals [3].

Future experiments will employ immunocytochemistry to explore the effects of mGluR4a receptor expression and distribution in the basal ganglia of hemi-parkinsonian rhesus monkeys that have been treated with mGluR agonists. Such experiments will be completed over the course of the next 2 years as the monkeys are sacrificed upon the completion of Specific Aim 2. In addition, all of the same characterization and localization experiments will be performed for mGluR4b, the other isoform.

Specific Aim II.

Determine the effect of selective group III mGluR agonists and antagonists on synaptic transmission at the major excitatory synapses in the output nuclei of the basal ganglia.

Whole cell patch clamp techniques were used to record the electrophysiological effects of mGluR agonists and antagonists on synaptic transmission in the BG. In particular, the role of mGluRs in the SNr, STN, and GP were studied. Our previously reported studies showed that group III mGluRs mediate a presynaptic inhibition of EPSCs at the STN-SNr synapse[4], and also mediate a reduction in IPSC amplitude in the SNr [5].

We have also shown that two group III mGluRs (mGluR4 and mGluR7) are presynaptically localized on striatal terminals in the GP, where they could reduce GABA release[6], and that activation of the group III mGluRs inhibits both GABAergic and glutamatergic transmission in the SNr[7,8].

In the course of our studies on the roles of mGluR4, we perform routine controls for the effects of other mGluRs. Interestingly, we have found that both group I and group II mGluRs play powerful roles in regulation of basal ganglia output. Group II mGluRs were found to mediate a presynaptic reduction of EPSCs in the SNr. Consistent with this finding is the observation that the highly selective group II mGluR agonist LY354740 reverses catalepsy in a rodent model of PD[4] [9].

Activation of group I mGluRs produces a reduction in IPSC amplitude and directly excites SNr projection neurons [5,10,11,12,]. These findings suggest that activation of group I mGluRs can excite GABAergic projection neurons both by direct stimulation and by disinhibition. Immunocytochemical analysis at the light and electron microscopic levels reveal that both mGluRs 1 and 5 are localized post-synaptically at putative glutamatergic synapses in the SNr. Therefore, to determine which mGluR mediates this response, we have performed a more thorough characterization of the pharmacology of this effect. Interestingly, mGluR1 appears to be the sole mediator of the group I mediated depolarization of inhibitory GABAergic neurons in the SNr. A role for mGluR5 has not yet been established. Light presynaptic staining for mGluR1 was also observed at asymmetric synapses. Consistent with this, activation of presynaptic group I mGluRs decreases inhibitory transmission in the SNr. The combination of excitation and disinhibition induced by group I mGluR activation could lead to a large excitation of the SNr projection neurons. This is likely to play an important role in the powerful excitatory control that the STN exerts on the BG output neurons. Based on this, it is possible that selective group I mGluR antagonists could provide therapeutic benefits for patients suffering from PD [11, 12]

In addition to direct excitatory actions in the SNr, mGluRs might alter the activity of the SNr by modulating the primary source of glutamatergic excitation, the STN. Therefore, the role of mGluRs in modulation of excitatory and inhibitory synaptic transmission in the STN was examined. Stimulating electrodes were placed in the internal capsule (IC) for stimulating descending afferents and in the cerebral peduncle for stimulating ascending afferents. EPSCs were elicited in the STN in the presence of 10μM Bicuculline, and IPSCs were elicited in the presence of 20μM CNQX and 20μM L-AP4. Under IC stimulation, the group I

selective mGluR agonist DHPG (100μM) caused a 34.3±3.3% reduction of EPSCs, the group II agonist LY354740 (100nM) caused a 43.5±6.8% reduction in EPSCs, and the group III agonist L-AP4 (1mM) caused a 80.9±6.7% reduction in EPSCs. We are currently investigating this mGluR inhibition of EPSCs to determine whether this effect is due to a pre- or a post-synaptic modulation [13].

Finally, the functional roles of group I mGluRs was investigated in the two most predominant cell types found in the GP. Group II and Group III mGluR agonists have no effect on membrane potential in type II GP neurons. DHPG, a group I mGluR selective agonist, causes a robust depolarization in both type I and type II GP neurons. MPEP, a mGluR5 antagonist caused an increase in DHPG-mediated depolarization, and induced oscillations in membrane potential in type II GP neurons. The mGluR1 antagonist, LY367385, suppressed DHPG-mediated depolarization in type II GP neurons[13].

Additional studies will provide a complete characterization of the electrophysiological properties of mGluRs in the BG. The ability of groups II and III mGluRs to modulate the excitatory transmission of glutamate at the STN-SNr synapse, combined with the data obtained on the ability of group I mGluRs to excite BG output neurons indicates that the development of novel therapeutic agents that target the specific receptors at these synapses could provide relief from the symptoms of PD.

Specific Aim III.

To evaluate the therapeutic potential of group III agonists in hemi-parkinsonian monkeys.

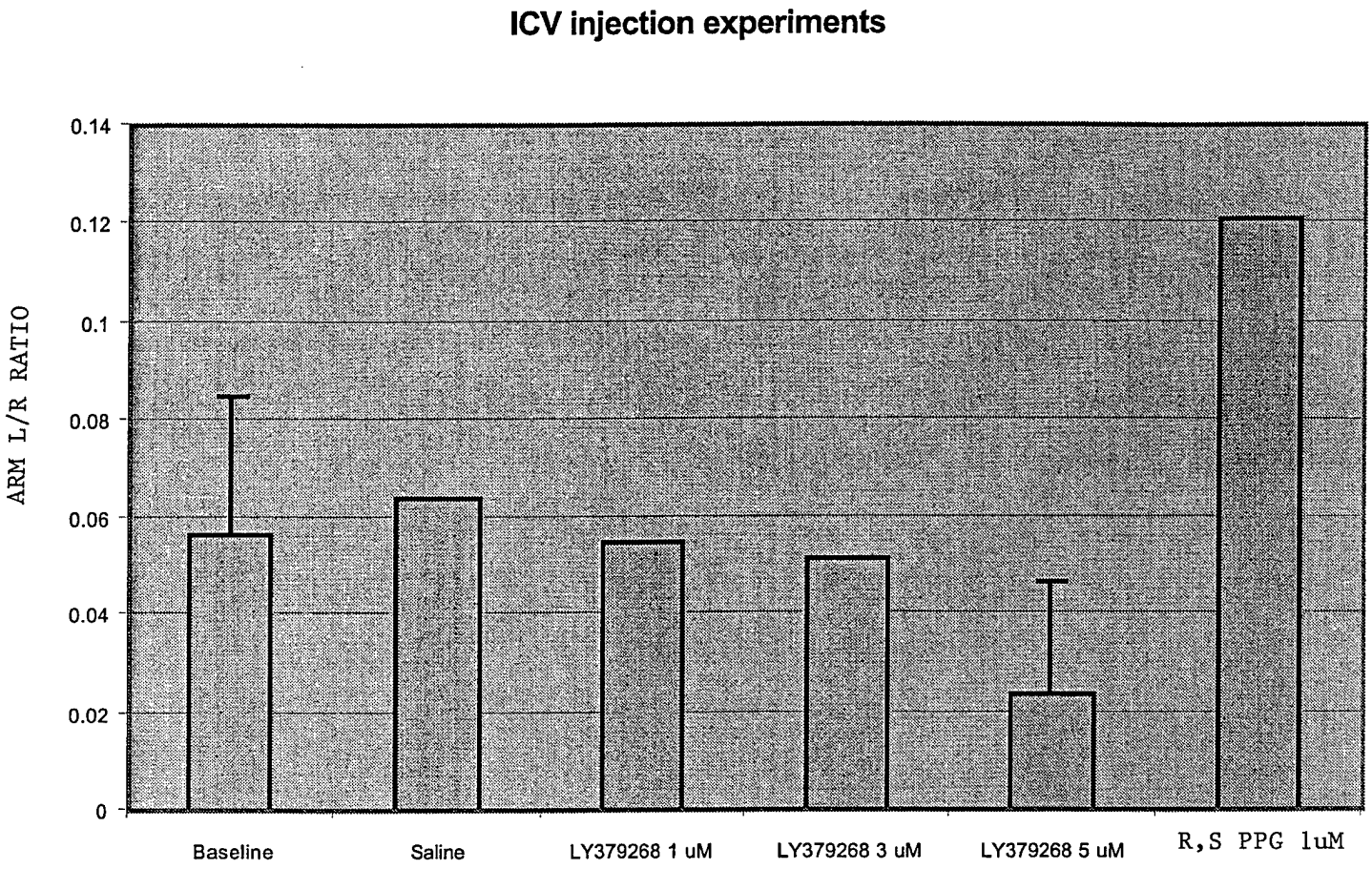
This aim directly measures the efficacy of mGluR agonists on hemi-parkinsonian monkeys. These experiments are technically challenging, and progress has been slow relative to our progress on tehj other two aims.are technical. In order to achieve a hemiparkinsonian state, the animals had to receive multiple MPTP injections followed by a fairly lengthy observation period to document the presence of parkinsonian signs and possible recovery. While such difficulties are unavoidable in this type of experiment, we have made some progress in testing for therapeutic efficacy of group II agonists. Overall, two rhesus monkeys were behaviorally conditioned to tolerate transfers from their home cages into the primate chair. We then carried out baseline observations using an automated activity monitoring cage with eight sets of infrared beams. Activity is being measured by counting infrared (IR) beam crossings over a 20-minute period. The pattern of IR crossings is

stored on computer disk, and can be used to later analyze behavioral patterns such as rotational behaviors, the amount of time spent in the upright posture, etc. In addition, we used a computer-assisted behavioral observation method, by which an observer scores movements of individual limbs of the animal by pressing keys on a computer keyboard. A computer stores the timing and length of key presses. More than one key can be scored at any given time. This allows for the calculation of the ratio between the left and right arm movements and allows for the normalization of the data with respect to the left (Parkinsonian) side. An increase in the L/R ratio indicates improvements in parkinsonism. Finally, a behavioral rating scale was completed on each experimental day, scoring the presence or absence of parkinsonian motor signs, and dyskinetic movements/stereotypes. After the initial behavioral observations, the monkeys were each treated with a single injection of MPTP (0.4 mg/kg) into their right internal carotid artery, following published protocols. After a stable parkinsonian state was documented with the above-mentioned behavioral observation methods, the animals were fitted with steel recording chambers directed at the GPi and SNr/STN to carry out intracerebral injections. In one animal the SNr/STN chamber was positioned to also give access to the ventricular system for i.c.v. injections.

The group II mGluR agonist LY354740 (100 μM) injected in GPi resulted in a slight increase in the L/R ratio in one hemi-parkinsonian monkey. The same agonist produced no change in the overall activity when injected into the STN, and in the cerebral ventricular system (i.c.v.). Both subcutaneous and intramuscular injection of this drug induced vomiting, but no reasonable antiparkinsonian effects. The group I antagonist MPEP (2mmol/L) and the group II mGluR antagonist LY341495 (1 mmol/L) had no effect when injected into the STN.

Interestingly, however, the i.c.v. administration of group III agonist R,S PPG (1 μM) resulted in substantial antiparkinsonian effects, with an almost two-fold increase of the arm L/R ratio baseline (Fig. 1). Future plans involve repeating this experiment and testing the effects of different doses of R,S PPG. We will also measure the effects of combining a group I antagonist with group III agonists.

Figure 1 . Comparison of the antiparkinsonian effects of i.c.v. injections of mGluR active compounds in a hemiparkinsonian rhesus monkey, as expressed by the arm L/R ratio. LY379268 is a group II mGluR agonist R,S PPG is a group III mGluR agonist.



KEY RESEARCH ACCOMPLISHMENTS

- Anatomy:
  1. Antibodies to mGluR4a were developed, fully characterized, and used to describe the anatomical distribution and localization of mGluR4a in rat brain.
  2. Antibodies to group I mGluRs were thoroughly characterized and used to demonstrate their post-synaptic localization in the SNr.

- Electrophysiology:
  1. Group III mGluRs mediate a presynaptic inhibition of EPSCs at the STN-SNr synapse.
  2. Group III mGluRs mediate a reduction in IPSC amplitude in the SNr.
  3. Group II mGluRs mediate a presynaptic reduction of EPSCs in the SNr.
  4. Group II agonist LY354740 reverses catalepsy in an animal model of PD.
  5. Activation of group I mGluRs produces a reduction in IPSC amplitude and directly excites SNr projection neurons.
  6. Group I mGluRs, particularly mGluR5, mediate the depolarization of STN by both excitation and disinhibition of output projection neurons.

- Behavior:

  Two rhesus monkeys have achieved a stable hemi-parkinsonian state. They were behaviorally trained to tolerate transfers from their home cage into a primate chair. Initial observations were carried out to quantify the parkinsonian symptoms. They were given various intracerebral and intraventricular injections of mGluR agonists and antagonists to measure the efficacy of these drugs on the symptoms of parkinsonism.

REPORTABLE OUTCOMES

Book Chapters

1. Marino, Michael J., *et al.* Localization and Physiological Roles of Metabotropic Glutamate Receptors in the Indirect Pathway. In Movement Disorders, M. DeLong, A.M. Graybiel, S.T. Kitai, eds. *(In Press)* (2000).

2. Conn, *et al.* Physiological roles of multiple metabotropic glutamate receptor subtypes in the rat basal ganglia. In Basal Ganglia and Thalamus in Health and Movement Disorders, I. Ilinsky ed. *(In Press)* (2000).

Papers and Manuscripts

1. Bradley, S.R., *et al.*, Activation of Group II metabotropic glutamate receptors inhibits synaptic excitation of the substantia nigra pars reticulata. *Journal of Neuroscience,* 2000. **20**(9): p.3085-3094.
2. Marino, M.J., Marion Wittmann, Stefania Risso Bradley, George W. Hubert, Yoland Smith, and P. Jeffrey Conn. Activation of Group I Metabotropic Glutamate Receptors Produces a Direct Excitation and Disinhibition of GABAergic Projection Neurons in the Substantia Nigra Reticulata. *Submitted Journal of Neuroscience* (2000).
3. Wittmann, M., Michael J Marino, Stefania Risso Bradley, and P. Jeffrey Conn. Activation of Group III Metabotropic Glutamate Receptors Inhibits GABAergic and Glutamatergic Transmission in the Substantia Nigra Pars Reticulata. *Submitted Journal of Neurophysiology* (2000).
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6. Bradley, S.R., *et al.*, Immunohistochemical localization of subtype 4a metabotropic glutamate receptors in the rat and mouse basal ganglia. *J Comp Neurol*, 1999. **407**(1): p. 33-46.

ABSTRACTS

1. Awad, H. and P. Conn, Physiological actions of metabotropic glutamate receptors in neurons of the subthalamic nucleus. *Neuropharmacology Abstr.*, 1999. **38**(10). A3
2. Bradley, S.R., *et al.*, Physiological roles of presynaptically localized type 2, 3 and 7 metabotropic glutamate receptors in rat basal ganglia. *Soc. Neurosci. Abstr.*, 1999. **25**(Abstr. No. 176.16).
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CONCLUSIONS

The Group III receptor, mGluR4a is found in the STR, SNr, and EPN, key basal ganglia structures involved in the manifestation of Parkinsonian symptoms. The globus pallidus GP contains a high concentration of presynaptic mGluR4a neuronal fibers that project from the striatum. This data supports the idea that mGluR4a may be a potential target in the treatment of PD.

The electrophysiological studies showed that group III mGluR agonists mediate a presynaptic inhibition of EPSCs at the STN-SNr synapse and a reduction in IPSC amplitude in the SNr. Group II mGluRs mediate a presynaptic reduction of EPSCs in the SNr. The Group II agonist LY354740 reverses catalepsy in an animal model of PD. Activation of Group I mGluRs produces a reduction in IPSC amplitude and directly excites SNr projection neurons. Group I mGluRs are mostly postsynaptic in the basal ganglia. Activation of postsynaptic Group I mGluRs in the STN may provide a powerful site for the treatment of PD due to the ability of these neurons to be simultaneously excited and disinhibited. The ability of group II and group III mGluRs to modulate the excitatory transmission of glutamate at the STN-SNr synapse, combined with the data obtained on the ability of group I mGluRs to excite BG output neurons and the anatomical findings all provide compelling evidence that the development of novel therapeutic agents that target the specific receptors at these synapses could provide relief from the symptoms of PD. Continuing studies will be essential to further define the roles of mGluR4 and important related mGluRs in basal ganglia function, and most critically, their value as targets for novel therapeutic drugs in hemi-parkinsonian monkeys.

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APPENDICES

I. Wittmann, M., Michael J Marino, Stefania Risso Bradley, and P. Jeffrey Conn. Activation of Group III Metabotropic Glutamate Receptors Inhibits GABAergic and Glutamatergic Transmission in the Substantia Nigra Pars Reticulata. *Submitted Journal of Neurophysiology* (2000).

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X. Marino, M.J., Wittmann, M., Bradley, S.R., Conn, P.J. Metabotropic Glutamate Receptor-Mediated Regulation of Excitatory Transmission in the Rat SNr. *International Basal Ganglia Society Abstract*

XI. Marino, M. J., Wittmann, M., Conn, P. J. Dopamine Regulation of Metabotropic Glutamate Receptor Signaling in the Rat Substantia Nigra Pars Reticulata. *Abstract, American College of Neuropharmacology Annual Meeting* (2000).

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XIII. Awad, H., *et al.,* Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *Journal of Neuroscience*, 2000. **20**(21): p. 7871-7879.

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Activation of Group III Metabotropic Glutamate Receptors Inhibits GABAergic and Glutamatergic Transmission in the Substantia Nigra Pars Reticulata.

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The GABAergic projection neurons of the substantia nigra pars reticulata (SNr) exert an important influence on the initiation and control of movement. The SNr is a primary output nucleus of the basal ganglia (BG) and is controlled by excitatory inputs from the subthalamic nucleus (STN) and inhibitory inputs from the striatum and globus pallidus. Changes in the output of the SNr are believed to be critically involved in the development of a variety of movement disorders. Anatomical studies reveal that metabotropic glutamate receptors (mGluRs) are highly expressed throughout the BG. Interestingly, mRNA for group III mGluRs are highly expressed in STN, striatum and globus pallidus and immunocytochemical studies have shown that the group III mGluR proteins are present in the SNr. Thus, it is possible that group III mGluRs play a role in the modulation of synaptic transmission in this nucleus. We performed whole cell patch clamp recordings from nondopaminergic SNr neurons to investigate the effect of group III mGluR activation on excitatory and inhibitory transmission in the SNr. We report that activation of group III mGluRs by the selective agonist L-AP4 (100 μM) decreases inhibitory synaptic transmission in the SNr. Miniature IPSC studies and paired-pulse studies reveal that this effect is mediated by a presynaptic mechanism. Furthermore we found that L-AP4 (500 μM) also reduces excitatory synaptic transmission at the STN-SNr synapse by action on presynaptically localized group III mGluRs. The finding that mGluRs modulate the major inputs to SNr neurons suggests that these receptors may play an important role in motor function and could provide new targets for the development of pharmacological treatments of movement disorders.

Introduction

The basal ganglia (BG) is a highly interconnected group of subcortical nuclei in the vertebrate brain that plays a critical role in control of movement. The substantia nigra pars reticulata (SNr) is an important component of the basal ganglia motor circuit. The GABA containing projection neurons of the SNr together with those of the entopeduncular nucleus comprise the principal output nuclei of the BG (Grofova et al. 1982) which exert an important influence on the initiation of movement (Kilpatrick et al. 1982) and on motor control (Alexander and Crutcher 1990). Because of this, changes in the GABAergic output of the BG are believed to play an important role in physiological as well as in pathophysiological conditions.

Inhibitory output from the SNr is controlled by two opposing but parallel pathways (DeLong 1990; Bergman et al. 1990). The "direct pathway" originates from a subpopulation of GABAergic striatal neurons that project directly to the SNr and thereby inhibit activity in these output neurons. The "indirect pathway" originates from a different population of GABAergic striatal neurons that project to the SNr via the external segment of the globus pallidus and the subthalamic nucleus (STN) providing an excitatory glutamatergic input to SNr neurons. An intricate balance of activity between these pathways is believed to be necessary for a normal fine tuning of motor function, and the disruption of this balance leads to various movement disorders (Wichmann and DeLong 1997; Wichmann and DeLong 1998). Hypokinetic movement disorders such as Parkinson's disease are produced by a relative increase in BG output mediated by a decrease in activity of inhibitory inputs via the direct pathway and an increase in activity of excitatory inputs through the indirect pathway. A relative decrease of BG output, on the other

hand leads to the development of hyperkinetic disorders including Huntington's disease and Tourette syndrome. Furthermore, inhibition of GABAergic SNr projection neurons has been shown to result in suppression of seizures in various animal models of epilepsy (Deransart et al. 1998). Since the output of the SNr is so critically involved in normal as well as pathological brain processes, receptors that modulate excitatory and inhibitory inputs to SNr neurons could provide important targets for drug development. One family of receptors that may provide such a target are the metabotropic glutamate receptors (mGluRs).

Metabotropic glutamate receptors are G-protein coupled receptors that are highly expressed throughout the BG (Testa et al. 1994, 1998; Kerner et al. 1997; Kosinski et al. 1998, 1999; Bradley et al. 1999a, 1999b). Behavioral and physiological studies have shown that mGluRs play important roles in regulation of BG function. To date, eight mGluR subtypes (mGluR1-8) have been cloned, and are classified into 3 major groups based on sequence homology, coupling to second messenger systems, and selectivities for various agonists (Conn and Pin 1997). Group I mGluRs (mGluR1, and 5) couple to G_q and activation of phosphoinositide hydrolysis, while group II mGluRs (mGluR2, and 3) and group III mGluRs (mGluR4, 6, 7 and 8) couple to $G_{i/o}$ and associated effector systems such as adenylyl cyclase. The mGluRs (with the exception of mGluR6) are widely distributed throughout the central nervous system and play important roles in regulating cell excitability and synaptic transmission at excitatory and inhibitory synapses.

We have previously shown that presynaptically localized group II mGluRs inhibit glutamatergic transmission at the STN-SNr synapse and therefore can reduce pathological conditions of overexcitation of GABAergic SNr neurons providing a useful approach for the treatment of Parkinson's disease (Bradley et al. 2000). Furthermore, we have shown that

postsynaptically localized group I mGluRs produce a direct excitation of GABAergic SNr neurons (Marino et al. 1999, 2000). Interestingly, recent immunocytochemical studies reveal that group III mGluRs are also present in the SNr (Kosinski et al. 1999; Bradley et al. 1999b). However, the physiological roles of group III mGluRs in the SNr are not known. We now report that activation of group III mGluRs decreases transmission at inhibitory and excitatory synapses onto nondopaminergic, presumably GABAergic, SNr neurons and that these effects are mediated by presynaptic mechanisms.

Methods

Materials

Bicuculline, 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), (RS)-α-Cyclopropyl-4-phosphonophenylglycine (CPPG), D(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), L(+)-2-Amino-4-phosphonobutyric acid (L-AP4), and L-Serine-O-phosphate (L-SOP) were obtained from Tocris (Ballwin, MO). 2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl) propanoic acid (LY341495) was a gift from D. Schoepp and J. Monn (Eli Lilly, Indianapolis, IN). All other materials were obtained from Sigma (St. Louis, MO).

Electrophysiology

Whole-cell patch clamp recordings were obtained under visual control as previously described (Marino et al. 1998; Bradley et al. 2000). 15-18 day old Sprague-Dawley rats were used for all patch clamp studies. Some animals were transcardially perfused with an ice cold sucrose buffer (in mM: Sucrose, 187; KCl, 3; $MgSO_4$, 1.9; KH_2PO_4, 1.2; Glucose, 20; $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2). While this tended to increase slice viability it did not have any effect on experimental outcome. Therefore, data from perfused and non-perfused animals have been pooled. Brains were rapidly removed and submerged in ice cold sucrose buffer. Parasaggital slices (300μm thick) were made using a Vibraslicer (WPI). Slices were transferred to a holding chamber containing normal ACSF (in mM: NaCl, 124; KCl, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.0; $CaCl_2$, 2.0, Glucose, 20; $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2). In all experiments, 5 μM glutathione and 500 μM pyruvate were included in the sucrose buffer and

holding chamber. Slices were transferred to the stage of a Hoffman modulation contrast microscope and continually perfused with room temperature ACSF (~3ml/min, 23-24^{0}C). Neurons in the substantia nigra pars reticulata were visualized with a 40X water immersion lens. Patch electrodes were pulled from borosilicate glass on a Narashige vertical patch pipette puller and filled with (in mM: potassium gluconate, 140; HEPES, 10; NaCl, 10; EGTA, 0.6; NaGTP, 0.2; MgATP, 2; pH adjusted to 7.4 with 0.5 N KOH). Electrode resistance was 3-7 MΩ. For measurement of synaptically evoked currents , bipolar tungsten electrodes were used to apply stimuli.

Nondopamiergic, presumably GABAergic, SNr neurons were identified according to previously established electrophysiological criteria (Richards et al. 1997). Nondopaminergic neurons exhibited spontaneous repetitive firing, short duration action potentials , little spike frequency adaptation, and a lack of inward rectification, while dopaminergic neurons displayed no, or low frequency spontaneous firing, longer duration action potentials, strong spike frequency adaptation, and a pronounced inward rectification. All of the data presented in these studies are from neurons which fit the electrophysiological criteria of nondopaminergic, neurons

Measurement of inhibitory and excitatory postsynaptic currents (IPSCs/EPSCs):

IPSCs were evoked with the stimulation electrode placed within the SNr rostrally or caudally to the recorded cell outside the cerebral peduncle and recorded at a holding potential of -50 mV. CNQX (10-20 μM) and D-AP5 (10-20 μM) were present in the bath to block excitatory transmission. To study miniature IPSCs (mIPSCs) the 140 mM potassium gluconate in the internal solution were substituted with 140 mM CsCl to reduce postsynaptic mGluR effects and

increase current amplitude. Therefore, outward mIPSCs were recorded at a holding potential of -80 mV in the presence of 1 μM tetrodotoxin (TTX).

EPSCs were evoked with the stimulation electrode placed into the STN and recorded from a holding potential of -60 mV. Picrotoxin (50 μM) was bath applied during all EPSC recordings to block inhibitory transmission. For studies of mEPSCs, slices were bathed in standard ACSF with the addition of mannitol (50 mM), tetrodotoxin (500 nM), and bicuculline (10 μM) warmed to 25^{0}C. Miniature EPSCs were recorded from a holding potential of -80 mV. For measurement of kainate-evoked currents kainate (100 μM) was pressure ejected into the slice from a low resistance pipette as previously described (Marino et al. 1998; Bradley et al. 2000). Kainate-evoked currents were recorded from a holding potential of -60 mV, and slices were bathed in ACSF containing 500 nM tetrodotoxin TTX).

Results

Previous studies have shown that group III mGluRs are expressed in the SNr and in nuclei sending major inhibitory and excitatory projections to this structure (Bradley et al. 1999b; Kosinski et al. 1999). We therefore determined whether specific agonists of group III mGluRs have an effect on inhibitory or excitatory transmission in SNr neurons

Activation of group III mGluRs suppresses inhibitory synaptic transmission (IPSCs) in the SNr. Whole-cell patch clamp recordings were made from electrophysiologically identified nondopaminergic neurons of the SNr in midbrain slices. IPSCs were evoked by stimulating within the SNr with bipolar stimulation electrodes (0.4-12.0 μA, every 30 seconds) and were recorded at a holding potential of -50 mV in the presence of AMPA receptor (CNQX; 10-20 μM) and NMDA receptor (D-AP5; 10-20 μM) antagonists to block excitatory synaptic transmission. Bicuculline (10 μM; n=8; data not shown) abolished evoked IPSCs in all cells tested, confirming that the evoked currents were $GABA_A$ receptor-mediated responses.

Short (3 min) bath application of the group III mGluR selective agonist L-AP4 (100 μM) significantly reduced the amplitude of evoked IPSCs by 53.1 ± 4.7% (Figure 1A; $p<0.05$, n=9). This effect of L-AP4 was reversible (Figure 1B). Most experiments were performed at room temperature since increasing the temperature decreased slice viability. However, control experiments performed at 32°C revealed that L-AP4 also reduced IPSCs at higher temperatures (75.6 ± 10.5% inhibition, n=4). Concentration response analysis revealed that the inhibition of IPSCs by L-AP4 was concentration dependent. It furthermore suggested a biphasic effect with a

small response to concentrations between 1 μM and 10 μM and a more robust response at higher concentrations (Figure 1C).

To further pharmacologically characterize the effect of group III mGluR activation on GABAergic synaptic transmission in the SNr, we determined the effect of another group III mGluR-selective agonist and a group III mGluR-selective antagonist. The reduction of IPSC amplitudes induced by L-AP4 was mimicked by 1 mM L-SOP, another selective agonist for group III mGluRs (Figure 1D). Furthermore, the response to L-AP4 (100 μM) was completely blocked by a 10-15 min pre-incubation with the group II/III mGluR antagonist CPPG (500 μM) (Figure 1D) (Toms et al. 1996). Since we have previously shown that activation of group II mGluRs has no effect on inhibitory synaptic transmission in the SNr (Bradley et al. 2000), these data are consistent with the hypothesis that this response is mediated by activation of a group III mGluR.

The effect of group III mGluR selective agonists on IPSC amplitudes is mediated by a presynaptic mechanism. To examine the site of action of group III mGluR-selective agonists we determined the effect of a maximal concentration of L-AP4 on the amplitude of spontaneous miniature IPSCs (mIPSCs). All mIPSC recordings were preformed at a holding potential of -80 mV in the presence of CNQX (10-20 μM) and D-AP5 (10-20 μM) to block glutamatergic synaptic currents and 1 μM tetrodotoxin to block activity dependent release of transmitter. Miniature IPSCs were measured as inward currents with pipettes in which Cl^- (140 mM) was the major anion in the internal solution.

Application of the group III-selective agonist L-AP4 (500 μM) induced a significant

decrease in the frequency of mIPSCs (Figure 2A, $p<0.05$, n=4, t-test) while not affecting mIPSC amplitude (Figure 2A,B). Thus, L-AP4 induced a rightward shift of the inter-event interval cumulative probability plot but had no effect on the amplitude cumulative probability plot (Figure 2C). The average mIPSC frequency before drug application was 1.75 ± 0.16 Hz and 1.31 ± 0.06 Hz after application of 500 μM L-AP4 ($p<0.05$; n=4, t-test). The average mIPSC amplitude was 26.7 ± 4.1 pA before and 27.5 ± 3.3 pA after L-AP4 application ($p>0.05$; n=4, t-test). These findings are consistent with a presynaptic site of action for the group III mGluR mediated suppression of synaptic transmission. To further test this hypothesis, we also determined the effect of L-AP4 on paired-pulse facilitation of evoked IPSCs. All paired-pulse recordings were made in the presence of CNQX (10-20 μM) and D-AP5 (10-20 μM) with standard internal solution to allow measurement of outward IPSCs. IPSCs were evoked every 30 seconds by paired stimulations of equal strength with a 50 msec inter-pulse interval. At these intervals paired-pulse facilitation was observed in all recordings (Figure 3A, 148.4 ± 5.2 %, n=11). Only cells that showed an agonist induced effect on the amplitude of the first IPSC of at least 25% inhibition were used for analysis. Under these conditions L-AP4 (100 μM) induced an increase in the ratio of paired-pulse facilitation in 9 out of 10 cells (Figure 3). In these ten cells, the mean potentiation before drug application was 150.3 ± 5.4% and 194.5 ± 45.6 % in the presence of L-AP4 ($p<0.01$, n=10, two-tailed t-test). This represents an increase of paired-pulse facilitation induced by L-AP4 of 28.8 ± 7.3%. Taken together, these data suggest that L-AP4 reduces transmission at inhibitory synapses in the SNr by actions on presynaptic group III mGluRs, resulting in a reduction of GABA release.

Activation of group III mGluRs inhibits excitatory synaptic transmission (EPSCs) at the STN-SNr synapse. EPSCs were elicited by stimulation of the STN with bipolar stimulating electrodes (0.4-12.0 μA, every 30 seconds). All recordings were preformed at a holding potential of -60 mV in the presence of picrotoxin (50 μM) to block inhibitory synaptic transmission. EPSCs elicited with this protocol had a constant latency and were completely abolished with application of 10 μM CNQX (n=10, data not shown), suggesting that the synaptic response was a monosynaptic glutamatergic EPSC.

We have previously shown that activation of presynaptically localized group II mGluRs inhibits excitatory transmission at the STN-SNr synapse (Bradley et al. 2000). We now investigated the roles of group III mGluRs in regulating transmission at this synapse. Brief bath application of the group III mGluR selective agonist L-AP4 (500 μM) produced a significant depression of EPSCs in nondopaminergic SNr neurons (Figure 4A; $p<0.01$; n=6). This effect of L-AP4 was reversible (Figure 4B). The concentration response curve for L-AP4 revealed an EC_{50} of around 150 μM with a maximal effect of 72.9 ± 3.4% at a concentration of 500 μM L-AP4 (n=6, Figure 4C). As with the effect of L-AP4 on IPSCs, L-AP4 induced a similar effect when measured at 32^{0}C (78.8 ± 8.8%, n=3). L-SOP (1mM), another selective agonist for group III mGluRs, mimicked the effect of L-AP4 on EPSC amplitudes (Figure 4D). Furthermore, the response to L-AP4 was blocked by prior application (10-15 min) of the group II/III mGluR antagonist CPPG (500 μM) (Figure 4D) (Toms et al. 1996). To further insure that the effect of L-AP4 is mediated by activation of group III but not group II mGluRs we also investigated the concentration response relationship of the antagonist LY341495. This antagonist is selective for group II mGluRs but also blocks group III mGluRs at higher concentrations (Kingston et al.

1998). LY341495 blocked the effect of the group II mGluR selective agonist LY354740 with an IC_{50} value of approximately 30 nM. In contrast the IC_{50} value of LY341495 at blocking the response to L-AP4 was approximately 1 μM (Figure 4E). These values are consistent with the potencies of LY341495 at group II and group III mGluRs respectively. Taken together these data suggest that activation of group III mGluRs inhibits glutamatergic synaptic transmission at the STN-SNr synapse.

The effect of group III mGluR selective agonists on EPSC amplitudes is mediated by a presynaptic mechanism. To test the hypothesis that group III mGluRs mediate the depression of synaptic transmission at the STN-SNr synapse by a presynaptic mechanism we recorded spontaneous miniature EPSCs (mEPSCs) in the presence of tetrodotoxin (500 nM) to block activity-dependent release. All recordings were preformed at a holding potential of -80 mV and in the presence of bicuculline (10 μM) to block $GABA_A$-mediated synaptic currents.

Application of 500 μM L-AP4 had no significant effect on the amplitude or frequency of mEPSCs in SNr neurons (Figure 5A-C). The cumulative probability plot for inter-event intervals reveals a slight but non significant rightward shift. The average mEPSC frequency was 4.8 ± 1.5 Hz before drug application and 3.4 ± 0.9 Hz after drug application ($p>0.05$, n=5, t-test). Likewise, the cumulative probability plot of mEPSC amplitudes (Figure 5C) revealed that L-AP4 did not reduce mEPSC amplitude. The average mEPSC amplitude was 8.2 ± 1.1 pA before drug application and 7.3 ± 0.7 pA after drug application ($p>0.05$, n=5, t-test). To further determine the effect of L-AP4 on postsynaptic AMPA receptors we investigated the effects of maximal concentrations of L-AP4 on currents elicited by brief (50-500 msec) pressure ejection of the non-

selective AMPA/kainate receptor agonist kainic acid (100 μM) into the slice. Kainate application elicited a robust, stable, inward current in the presence of 500 nM tetrodotoxin in nondopaminergic SNr neurons (Figure 6A). Application of 500 μM L-AP4 had no significant effect on kainate-induced currents (Figure 6A-C), suggesting that L-AP4 does not modulate kainate-activated channels in SNr neurons.

The lack of an effect of L-AP4 on mEPSC amplitude and on kainate-evoked currents is consistent with a presynaptic site of action. In order to further test this hypothesis we determined the effect of L-AP4 on paired-pulse facilitation of evoked EPSCs. All paired-pulse recordings were performed at a holding potential of -60 mV in the presence of bicuculline (10 μM) and EPSCs were evoked by stimulating the cerebral peduncle every 20 seconds by paired stimulations of equal strength at 20-100 msec intervals. Stimulus strength and inter-pulse intervals were adjusted in each experiment so that the second EPSC was always greater in amplitude than the first (paired-pulse facilitation: 130.0 $\pm$ 6.5 %, n=7). L-AP4 (500 μM) reduced the absolute amplitude of EPSCs but also increased the ratio of paired-pulse facilitation significantly to 268 $\pm$ 35.0% (Figure 7, $p<0.01$, n=7, t-test). This represents an 105.9 $\pm$ 24.5% increase of facilitation induced by L-AP4. Taken together, these data provide strong support for the hypothesis that L-AP4 acts presynaptically to inhibit the evoked release of transmitter from glutamatergic terminals.

Discussion

The data presented in this study show that activation of group III mGluRs reduces GABAergic transmission in the SNr and that this reduction is mediated by a presynaptic mechanism. Furthermore, we present evidence that activation of presynaptically localized group III mGluRs inhibits excitatory synaptic transmission at the STN-SNr synapse.

All recordings in this study were from electrophysiologically identified nondopaminergic neurons in the SNr. The firing patterns of the cells included in this study, such as spontaneous repetitive firing, short duration action potentials, little spike frequency adaptation, and a lack of inward rectification, correspond to firing patterns reported for identified GABAergic SNr neurons *in vitro* (Richards et al. 1997). Furthermore, extracellular and intracellular recording studies *in vivo* show that the majority (ca 80%) of nondopaminergic cells in the SNr can be activated antidromically by thalamic or tectal stimulation (Guyenet and Aghajanian 1978, Grofova et al. 1982) indicating that the majority of nondopaminergic neurons in the SNr are projection neurons. Thus, it is likely that most of the neurons investigated in this study are GABAergic projection neurons, representing the major output neurons of the SNr. However, we cannot exclude the possibility that some of our results were obtained from GABAergic interneurons or other unidentified neuronal classes

Since it is known that a substantial proportion of inhibitory terminals onto SNr projection neurons arise from the striatum (Smith et al. 1998), it is possible that a significant portion of the L-AP4 induced effect is mediated by activation of group III mGluRs at striatonigral synapses, thereby acting on the direct pathway in the BG circuit. However, effects on other GABAergic

synapses can not be excluded since the GABAergic inputs to the SNr are heterogeneous. SNr projection neurons receive GABAergic inputs not only from the striatum but also from the globus pallidus, neighboring SNr projection neurons, and interneurons (Smith et al. 1998).

In our pharmacological studies we show that activation of group III mGluRs decreases GABAergic transmission in the SNr. Our findings that L-AP4 has no effect on mIPSC amplitude, and increases the ratio of paired-pulse facilitation provides strong evidence for a presynaptic mechanism. The relative high concentration of L-AP4 required to produce a maximal inhibition of IPSCs suggests that this effect is mediated by mGluR7 (Wu et al. 1998). These findings are in agreement with recent anatomical studies that indicate that the mGluR7 subtype is presynaptically localized to symmetric (inhibitory) synapses in the SNr (Kosinski et al. 1999). Interestingly, immunocytochemistry studies reveal that mGluR7 is presynaptically localized at both striatonigral and striatopallidal synapses (Kosinski et al. 1999) but mGluR4 appears to be more abundant at striatopallidal synapses than at striatonigral synapses (Bradley et al. 1999a). Taken together these data suggest that group III mGluRs may play important roles in the modulation of the BG circuit. While mGluR7 localization indicates this receptor subtype could modulate synaptic transmission in the direct as well as in the indirect pathway the subtype mGluR4 might more selectively modulate activity in the indirect pathway.

We have previously shown that activation of group II mGluRs inhibits excitatory synaptic transmission at the STN-SNr synapse (Bradley et al. 2000). We now demonstrate that activation of presynaptically localized group III mGluRs also inhibits synaptic transmission at this synapse. These findings are consistent with anatomical data demonstrating the presence of mGluR7 presynaptically localized at this synapse (Bradley et al. 1999c). The presynaptic mechanism of

action for L-AP4 at the STN-SNr synapse is suggested by three converging findings. First, L-AP4 has no significant effect on mEPSC amplitude. Second, L-AP4 did not reduce the response to exogenously applied kainic acid. Finally, L-AP4 enhanced paired-pulse facilitation. Taken together with anatomical studies demonstrating presynaptic localization of group III mGluRs on STN terminals (Bradley et al. 1999c), those data provide strong evidence that L-AP4 inhibits synaptic transmission by acting at a presynaptic site.

It is interesting that, while L-AP4 reduced both EPSCs and IPSCs by a presynaptic mechanism of action, activation of group III mGluRs had differential effects on the frequencies of mEPSCs and mIPSCs. Thus, L-AP4 induced a significant reduction in the frequency of mIPSCs but had no significant effect on the frequency of mEPSCs. This raises the possibility that L-AP4 might reduce excitatory and inhibitory synaptic transmission by different presynaptic mechanisms.

There are a number of potential mechanisms by which a receptor could act presynaptically to reduce action potential dependent release without decreasing the frequency of mEPSCs. For instance, mEPSCs are thought to be voltage independent and therefore should be insensitive to modulation of presynaptic voltage-dependent ion channels. If a receptor reduces transmission by inhibiting a presynaptic voltage-dependent calcium channel or increasing conductance through a voltage-dependent potassium channel rather than having some downstream effect on the release machinery, this may reduce evoked responses without affecting mEPSCs. This effect has been demonstrated at a variety of synapses where agents known to act presynaptically, such as the calcium channel blocker cadmium, abolish evoked EPSCs but have no effect on either the frequency or amplitude of mEPSCs (Parfitt and Madison 1993; Gereau and Conn 1995; Doze et al. 1995; Scanziani et al. 1995). If a receptor regulates synaptic transmission by a mechanism that

is downstream from the presynaptic calcium increase, this is more likely to lead to a decrease in mEPSC or mIPSC frequency. The present studies do not provide definitive insight into the precise mechanism by which L-AP4 reduces inhibitory and excitatory transmission in the SNr. However, the differential effects of L-AP4 on mEPSCs and mIPSCs may provide some important clues that could guide future studies.

In summary, these studies demonstrate that group III mGluR subtypes are involved in the modulation of both inhibitory and excitatory synaptic transmission in the SNr. These receptors therefore may provide exciting new targets for the development of pharmacological treatments of disorders that are believed to be caused by a shift in the balance of activity in the direct and the indirect pathway, such as Parkinson's disease, Huntington's disease, and Tourette syndrome. By selectively targeting different mGluR subtypes with specific mGluR agonists or antagonists it may be possible to restore the balance of activity in the BG circuit.

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Figure 1. Application of L-AP4 suppresses IPSCs in substantia nigra pars reticulata. (A) Example traces of evoked IPSCs before (Pre-Drug), during (L-AP4), and after (Washout) brief bath application of L-AP4. (B) Average time course of the effect of 100 μM L-AP4 demonstrating that the effect of L-AP4 on IPSCs is reversible. Each point represents the mean (± SEM) of data from 6 cells. (C) Dose-response relationship of L-AP4-induced suppression of IPSCs. The effect of inhibition of IPSCs shows an EC_{50} of around 20 μM. Each point represents the mean (± SEM) of 4 experiments. The effect of L-AP4 on IPSCs is mediated by group III mGluRs. (D) Bar graph showing the average effect of the selective agonists L-AP4 (100 μM) and L-SOP (1 mM) and the effect of the antagonist CPPG (500 μM) on the L-AP4 induced inhibition of IPSCs. Each bar represents the mean (± SEM) of data collected from 5 cells (*$p<0.01$, t-test).

Figure 2. Inhibition of IPSCs induced by the activation of group III mGluRs is mediated by a presynaptic mechanism. (A) Examples of mIPSC traces before (Pre-Drug) and during application of 500 μM L-AP4. (B) Amplitude histograms of mIPSCs before (left) and during application of 500 μM L-AP4 (right). (C) Cumulative probability plots showing the lack of an effect of L-AP4 on mIPSC amplitude (left) and a decrease in inter-event interval (right). Data shown are pooled data from 4 separate experiments.

Figure 3. L-AP4 increases the ratio of paired-pulse facilitation of evoked IPSCs. (A,B) Example traces of paired-pulse experiments before (A) and during application of 100 μM L-AP4 (B). (C) Overlay of the pre-drug trace (solid line) and a trace during application of L-AP4 scaled to the amplitude of the first IPSC (dashed line) is shown. L-AP4 increases the ratio of paired-pulse

facilitation in 9 out of 10 cells. (D) Bar graph showing the average effect of L-AP4 on the ratio of paired-pulse facilitation in those 10 cells. Each bar represents the mean (± SEM) of data collected from 10 cells (*$p<0.01$; two-tailed t-test).

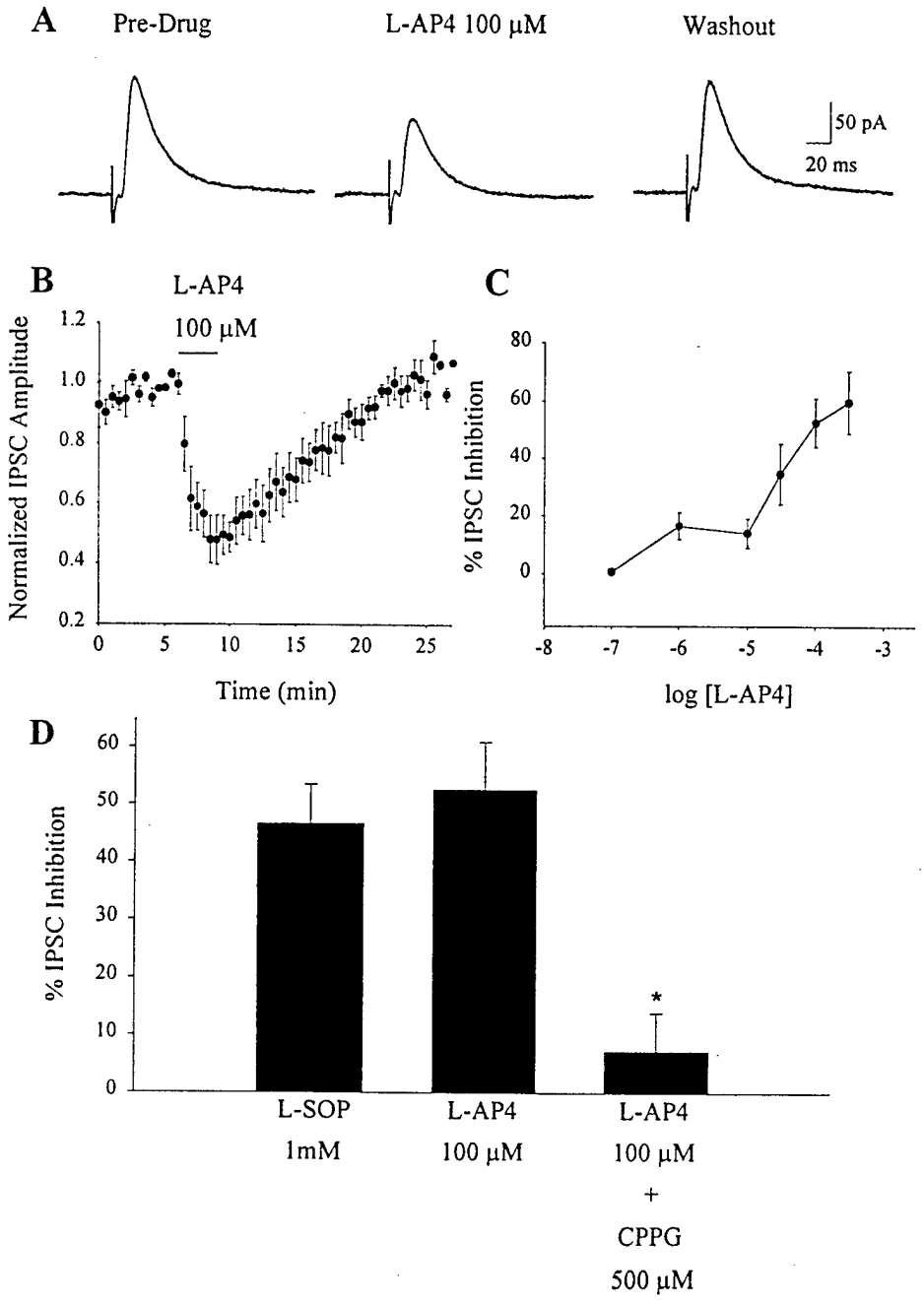
Figure 4. Application of L-AP4 suppresses EPSCs at the STN-SNr synapse by activation of group III mGluRs. (A) Example traces of evoked EPSCs before (Pre-Drug), during (L-AP4), and after (Washout) brief bath application of L-AP4. Application of L-AP4 dramatically reduces EPSCs in the SNr. (B) Average time course of the effect of 500 μM L-AP4 demonstrating that the effect of L-AP4 on EPSCs is reversible. Each point represents the mean (± SEM) of data from 3 cells. (C) Dose-response relationship of the L-AP4 induced inhibition of EPSCs. Each point represents the mean (± SEM) of data from 3 to 6 experiments. (D) Bar graph showing the average effects of group III mGluR selective agonists and the effect of the group II/III mGluR selective antagonist CPPG (500 μM) on the L-AP4 induced effect on EPSCs. Agonists include L-AP4 (500 μM) and L-SOP (1 mM). Each bar represents the mean (± SEM) of data collected from 5 cells (*$p<0.05$, t-test). (E) Concentration-response relationships of the group II/III mGluR selective antagonist LY341495. Each point represents the mean (±SEM) of data obtained from 3 cells.

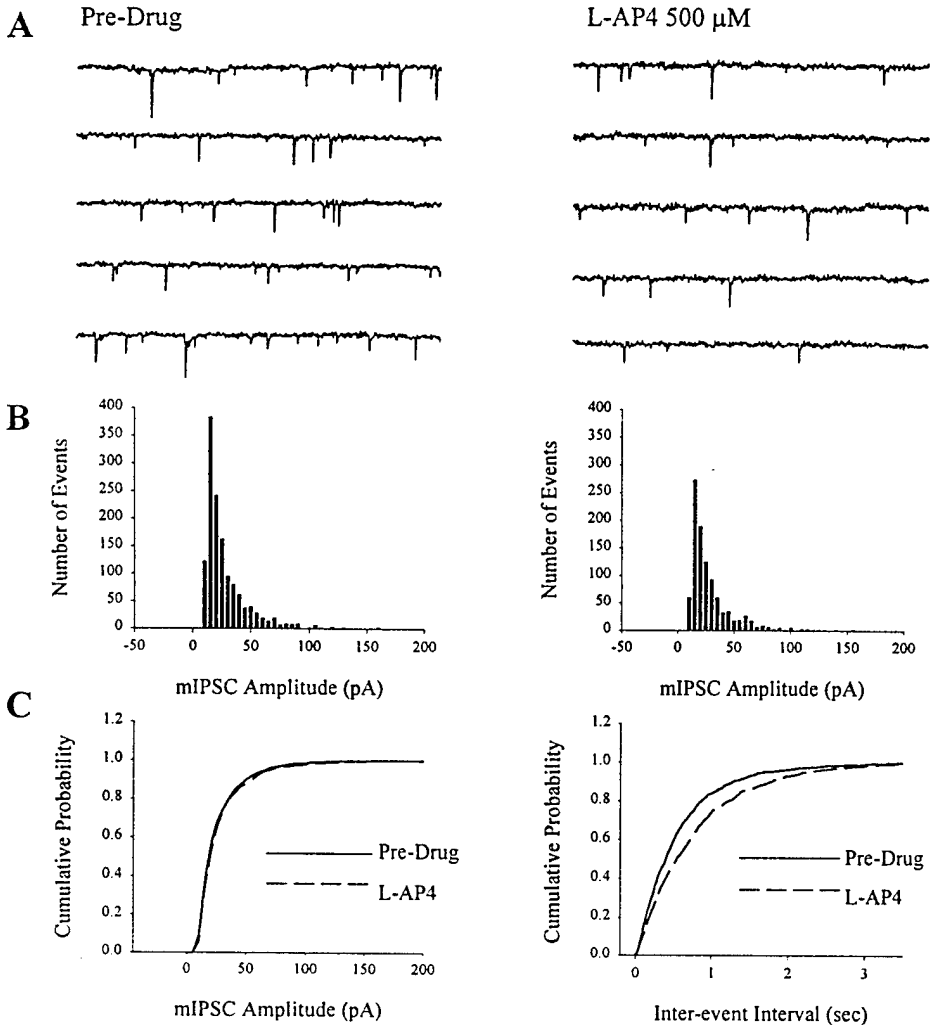
Figure 5. Inhibition of EPSCs induced by the activation of group III mGluRs is mediated by a presynaptic mechanism. (A) Examples of mEPSC traces before (Pre-Drug) and during application of 500 μM L-AP4. (B) Amplitude histograms of mEPSCs before (left) and during application of 500 μM L-AP4 (right). (C) Cumulative probability plots showing the lack of an effect of L-AP4

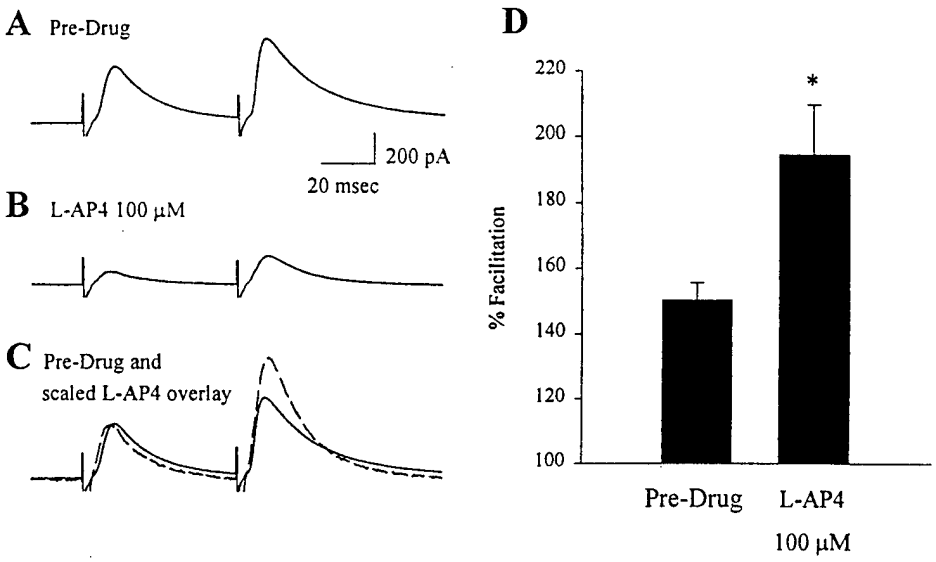
on mEPSC amplitude (left) and on inter-event interval (right). Data shown are pooled data from 5 separate experiments.

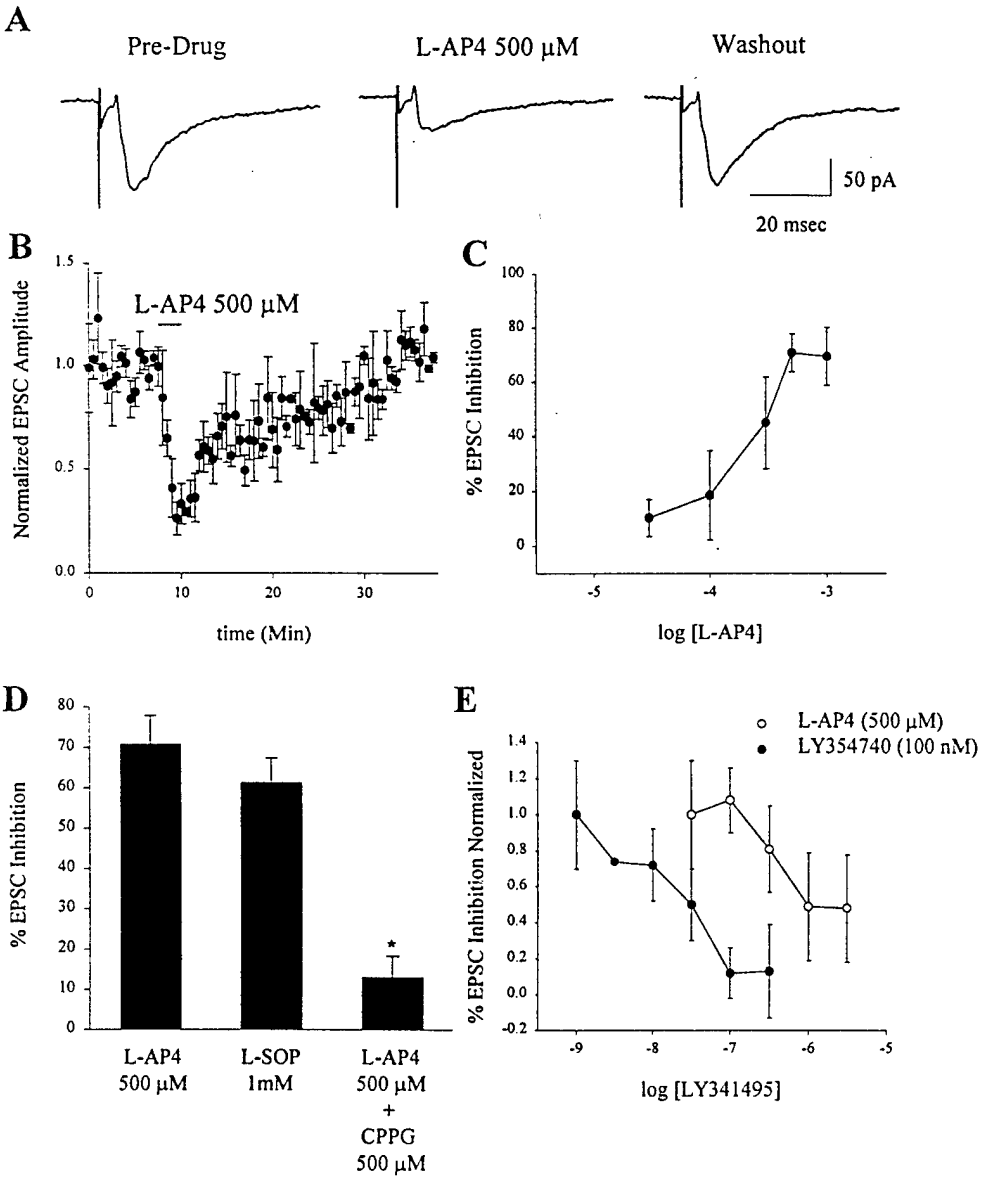
Figure 6. Activation of group III mGluRs does not alter the sensitivity of postsynaptic glutamate receptors in SNr neurons. (A) Representative traces of kainate-evoked currents in SNr projection neurons before (Pre-Drug) and during application of 500 μM L-AP4. (B) Time course of the effect of L-AP4 on the amplitude of kainate-evoked currents. (C) Bar graph showing the mean data demonstrating the lack of effect of group III mGluR activation on kainate-evoked currents. Each bar represents the mean (± SEM) of data collected from 5 cells ($p>0.05$, t-test).

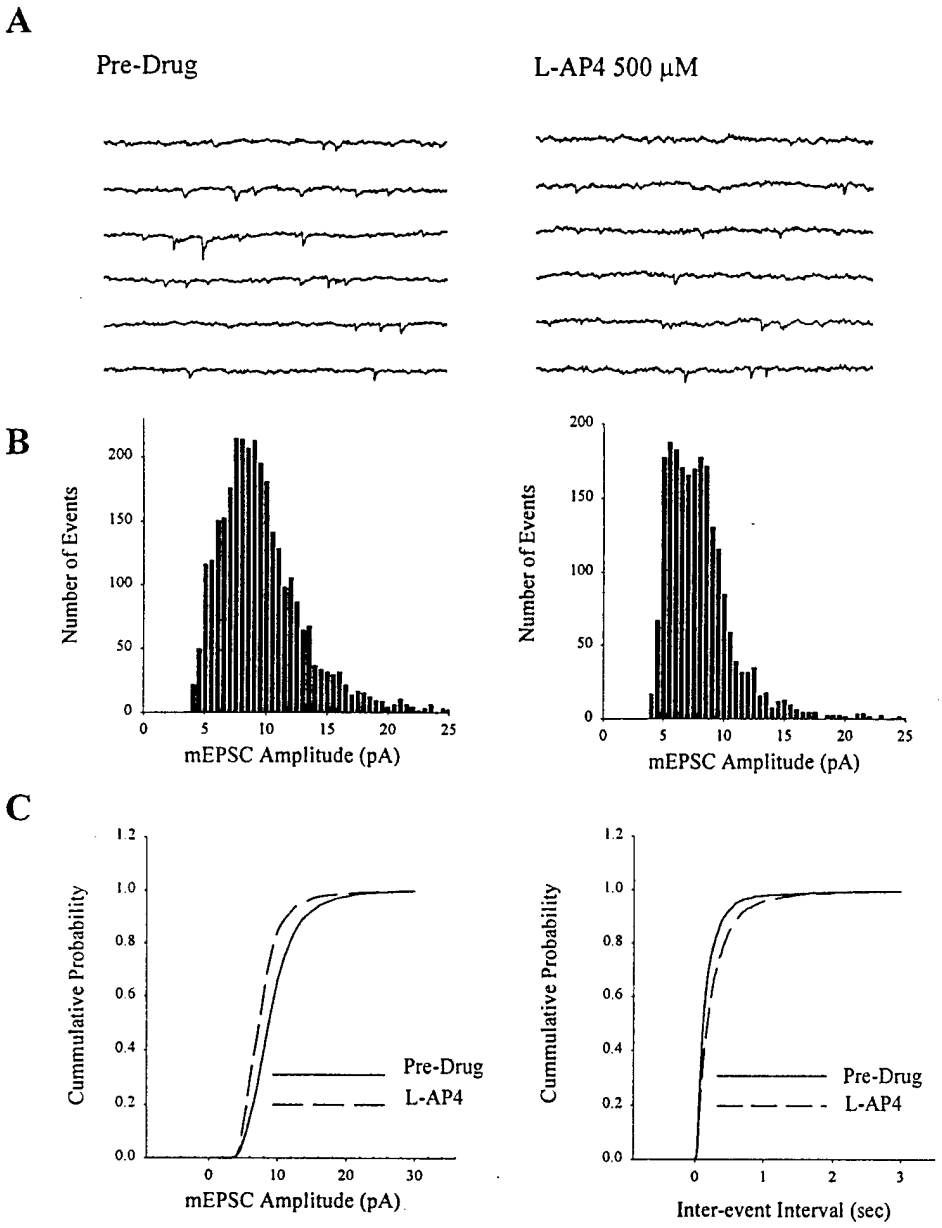
Figure 7. Activation of group III mGluRs increases the ratio of paired-pulse facilitation of evoked EPSCs. (A,B) Representative traces of paired-pulse facilitation before (pre-drug) and during application of 500 μM L-AP4. (C) Superimposed traces of pre-drug condition (solid line) and during application of L-AP4 (dashed line; trace scaled to the first EPSC of control condition). (D) Bar graph showing the average effect of L-AP4 on the ratio of paired-pulse facilitation. Each bar represents the mean (± SEM) collected from 7 cells (*$p<0.01$; two-tailed t-test).

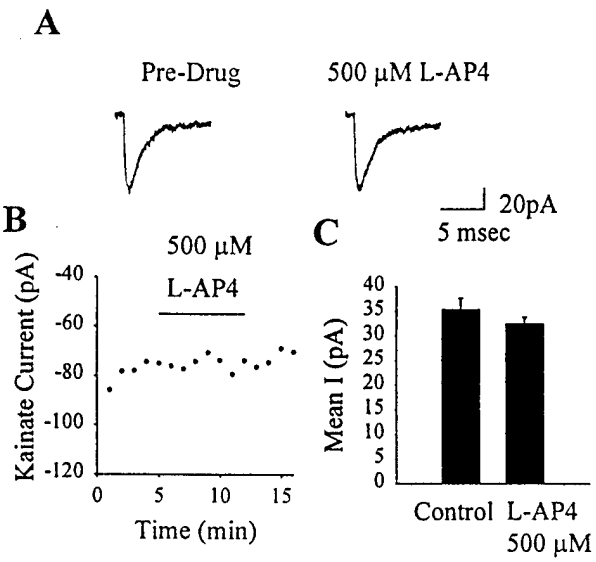

A
Pre-Drug
L-AP4 100 µM
Washout
50 pA
20 ms
B
L-AP4
100 µM
Normalized IPSC Amplitude
1.2
1.0
0.8
0.6
0.4
0.2
0
5
10
15
20
25
Time (min)
C
% IPSC Inhibition
80
60
40
20
0
-8
-7
-6
-5
-4
-3
log [L-AP4]
D
% IPSC Inhibition
60
50
40
30
20
10
0
*
L-SOP
1mM
L-AP4
100 µM
L-AP4
100 µM
+
CPPG
500 µM

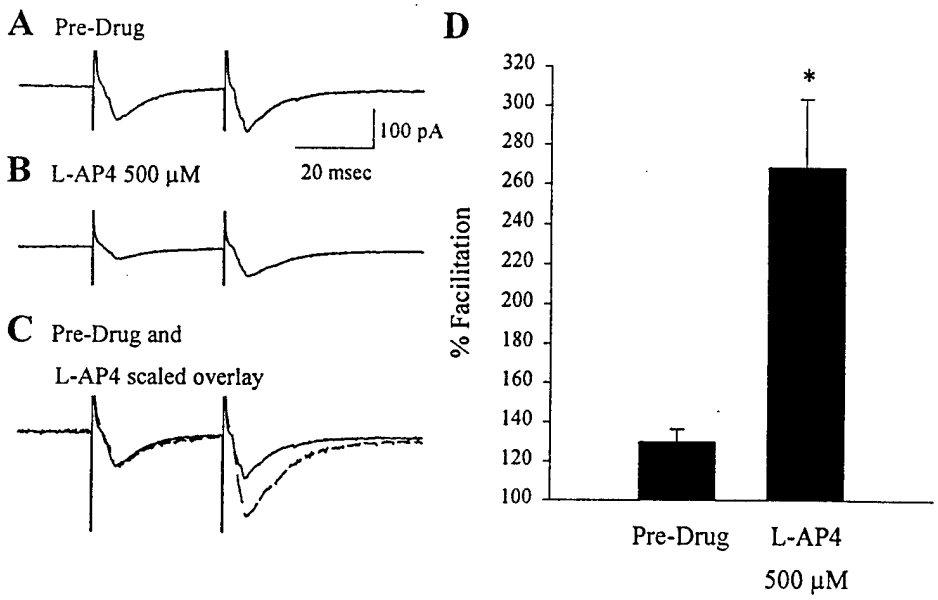

A
Pre-Drug
L-AP4 500 µM
B
Number of Events
mIPSC Amplitude (pA)
C
Cumulative Probability
Pre-Drug
L-AP4
mIPSC Amplitude (pA)
Inter-event Interval (sec)


A Pre-Drug
200 pA
20 msec
B L-AP4 100 µM
C Pre-Drug and
scaled L-AP4 overlay
D
220
200
180
160
140
120
100
% Facilitation
*
Pre-Drug
L-AP4
100 µM


A
Pre-Drug
L-AP4 500 µM
Washout
50 pA
20 msec
B
L-AP4 500 µM
Normalized EPSC Amplitude
time (Min)
C
% EPSC Inhibition
log [L-AP4]
D
% EPSC Inhibition
L-AP4 500 µM
L-SOP 1mM
L-AP4 500 µM + CPPG 500 µM
E
L-AP4 (500 µM)
LY354740 (100 nM)
% EPSC Inhibition Normalized
log [LY341495]


A
Pre-Drug
L-AP4 500 μM
B
Number of Events
mEPSC Amplitude (pA)
Number of Events
mEPSC Amplitude (pA)
C
Cummulative Probability
Pre-Drug
L-AP4
mEPSC Amplitude (pA)
Cummulative Probability
Pre-Drug
L-AP4
Inter-event Interval (sec)


A
Pre-Drug
500 µM L-AP4
20pA
5 msec
B
500 µM
L-AP4
Kainate Current (pA)
-40
-60
-80
-100
-120
0
5
10
15
Time (min)
C
Mean I (pA)
40
35
30
25
20
15
10
5
0
Control
L-AP4
500 µM


A Pre-Drug
100 pA
20 msec
B L-AP4 500 µM
C Pre-Drug and
L-AP4 scaled overlay
D
% Facilitation
320
300
280
260
240
220
200
180
160
140
120
100
*
Pre-Drug
L-AP4
500 µM

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Activation of Group I Metabotropic Glutamate Receptors Produces a Direct Excitation and Disinhibition of GABAergic Projection Neurons in the Substantia Nigra Pars Reticulata.

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ABSTRACT

A pathological increase in excitatory glutamatergic input to substantia nigra pars reticulata (SNr) from the subthalmic nucleus (STN) is believed to play a key role in the pathophysiology of Parkinson's disease (PD). We present an analysis of the physiological roles that group I metabotropic glutamate receptors play in regulating SNr function. Immunocytochemical analysis at the light and electron microscopic levels reveal that both mGuR1 and mGluR5 are localized postsynaptically at putative glutamatergic synapses in the SNr. Consistent with this, activation of group I mGluRs depolarizes substantia nigra GABAergic neurons. Interestingly, although both group I mGluRs (mGluR1 and mGluR5) are expressed in these neurons, the effect is mediated solely by mGluR1. Light presynaptic staining for mGluR1 was also observed at some asymmetric synapses. Consistent with this, activation of presynaptic group I mGluRs decreases inhibitory transmission in the SNr. The combination of direct excitatory effects and disinhibition induced by activation of group I mGluRs could lead to a large excitation of the SNr projection neurons. This suggests that group I mGluRs are likely to play an important role in the powerful excitatory control that the STN exerts on basal ganglia output neurons. Based on this, it is possible that selective group I mGluR antagonists could provide therapeutic benefits for patients suffering from PD.

Key Words: Substantia Nigra Pars Reticulata, Group I Metabotropic Glutamate Receptors, Movement Disorders, Slow Excitatory Postsynaptic Current, Disinhibition

The basal ganglia are a richly interconnected group of subcortical nuclei involved in the control of motor behavior. The primary input nucleus of the basal ganglia is the striatum, and the primary output nuclei are the substantia nigra pars reticulata (SNr) and the internal globus pallidus (entopeduncular nucleus in non-primates). The striatum projects to these output nuclei both directly, providing an inhibitory GABAergic input, and indirectly through the external globus pallidus and the STN. The STN provides excitatory glutamatergic input to the SNr. A delicate balance between inhibition and excitation of the output nuclei, by the direct and indirect pathways respectively, is believed to be critical for the normal motor control, and disruptions in this balance are believed to underlie a variety of movement disorders (Wichmann and DeLong, 1997; Wichmann and DeLong, 1998)

While much effort has been directed at elucidating the basic connectivity of the direct and indirect pathways, less is known about the modulatory influence various transmitters may have in these structures. Interestingly, increasing evidence suggests that G-protein-coupled metabotropic glutamate receptors (mGluRs) may play an important role in the regulation of basal ganglia function. To date, eight mGluR subtypes (mGluR1-8) have been cloned, and are classified into 3 major groups based on sequence homology, coupling to second messenger systems, and selectivities for various agonists (see Conn and Pin, 1997 for review). Group I mGluRs (mGluR1, and 5) couple to Gq and phosphoinositide hydrolysis, while groups II (mGluR2, and 3) and III (mGluR4, 6, 7, and 8) couple to Gi/Go and related effector systems such as inhibition of adenylate cyclase. These metabotropic glutamate receptors are widely distributed throughout the central nervous system where they play important roles in regulating cell excitability and synaptic transmission.

Previous studies have shown that mGluRs are highly expressed throughout the basal ganglia (Testa *et al.*, 1994; Kerner *et al.*,1997; Kosinski *et al.*, 1998; Testa *et al.*, 1998; Kosinski *et al.*, 1999; Bradley *et al.*, 1999a, 1999b), and play important roles in the regulation of synaptic transmission in the SNr. For example, activation of presynaptic group II and III mGluRs inhibits excitatory transmission at the STN-SNr synapse (Bradley *et al.*, 1999c, 2000) . One of the predominate postsynaptic effects of mGluRs in many brain regions is a group I mGluR-mediated depolarization (Guerineau *et al.*, 1994; Crepel *et al.*, 1994; Guerineau *et al.*, 1995; Miller *et al.*, 1995; Gereau and Conn, 1995a). Since glutamatergic innervation of the SNr from the STN plays an important role in motor control, an understanding of the roles mGluRs play in modulating SNr GABAergic neurons could provide important insight into the mechanisms involved in the regulation of SNr firing in both physiological and pathological states. We now report that activation of group I mGluRs produces an excitation of the SNr by two distinct mechanisms. Activation of postsynaptic mGluR1 induces a pronounced excitation of SNr GABAergic neurons that is mimicked by stimulation of excitatory afferents. In addition, activation of both mGluR1 and mGluR5 produce a decrease in inhibitory transmission in the SNr, resulting in increased excitability of this crucial basal ganglia output nucleus.

Materials and Methods

[R-(R*,S*)]-6-(5,6,7,8-Tetrahydro-6-methyl-1,3-dioxolo[4,5-g]isoquinolin-5-yl)furo[3,4-e]-1,3-benzodioxol-8(6H)-one (Bicuculline), 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), (RS)-3,5-Dihydroxyphenylglycine (DHPG), (RS)-3-Amino-2-(4-chlorophenyl)-2-hydroxypropyl-sulphonic acid (2-Hydroxysaclofen), L(+)-2-Amino-4-phosphonobutyric acid (L-AP4), and (S)-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385) were obtained from Tocris (Ballwin, MO). (S)-(+)-2-(3'-carboxy-bicyclo[1.1.1]pentyl-)glycine (CBPG) was obtained from Alexis Corp. (San Diego, CA). (+)-2-aminobicyclo[3.1.0]-hexane-2,6-dicarboxylate monohydrate (LY354740) was a gift from D. Schoepp and J. Monn (Eli Lilly, Indianapolis, IN). Methylphenylethynylpyridine (MPEP), and 7-hydroxyiminocyclopropan-[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt) were gifts from R. Kuhn (Novartis, Basel, Switzerland). All other materials were obtained from Sigma (St. Louis, MO).

Immunocytochemistry

Two male Sprague-Dawley rats were deeply anesthetized with Ketamine (100mg/kg) and Dormitor (10 mg/kg) and transcardially perfused with cold, oxygenated Ringer's solution followed by 500ml of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (PB 0.1M, pH 7.4) followed by 300ml of cold PB. Next, the brain was removed from the skull and stored in phosphate buffered saline (PBS 0.01M, pH 7.4) before being sliced on a vibrating microtome into 60μm transverse sections. These sections were then treated with 1.0% sodium borohydride for 20 minutes and rinsed in PBS.

The sections were preincubated at room temperature in a solution containing 10% normal goat serum (NGS), 1.0% bovine serum albumin (BSA), and 0.3% Triton X-100 in PBS for one hour. They were then incubated overnight at room temperature in a solution containing primary antibodies raised against synthetic peptides corresponding to the C-terminus of either mGluR1a (Chemicon, Temecula, CA) or mGluR5 (Upstate, Lake Placid, New York) diluted at 0.5-1.0mg/ml in a solution containing 1.0% NGS, 1.0% BSA, 0.3% Triton X-100 in PBS. Next, the sections were rinsed in PBS and transferred for one hour at room temperature to a secondary antibody solution containing biotinylated goat-anti-rabbit IgGs (Vector, Burlingame, CA) diluted 1:200 in the primary antibody diluent solution. After rinsing, sections were put in a solution containing 1:100 avidin-biotin-peroxidase complex (ABC, Vector). The tissue was then washed in PBS and 0.05M Tris buffer before being transferred to a solution containing 0.01M imidazole, 0.0005% hydrogen peroxide, and .025% 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO) in Tris for 7-10 minutes. Sections were then mounted on gelatin-coated slides, dried, and coverslipped with Permount..

For electron microscope studies, the sections were treated with cryoprotectant for 20 minutes and transferred to a -80°C freezer for an additional 20 minutes. The sections were then thawed and treated with successively decreasing concentrations of cryoprotectant and finally PBS. The immunocytochemical procedure was the same as used for the light microscope, except that Triton X-100 was not used, and the incubation in the primary antibody was carried out at 4°C for 48 hours.

After DAB revelation, the sections were processed for the electron microscope. They were first washed in 0.1M PB for 30 minutes and then postfixed in 1.0% osmium tetroxide for 20

minutes. After rinsing in PB, the tissue was dehydrated by a series of increasing concentrations of ethanol (50, 70, 90, and 100%). Uranyl acetate (1.0%) was added to the 70% ethanol to enhance contrast in the tissue. Next, the sections were exposed to propylene oxide and embedded in epoxy resin (Durcupan, Fluka, Buchs, Switzerland) for 12 hours. They were then mounted on slides, coverslipped, and heated at 60°C for 48 hours.

Four blocks (2 for mGluR1a and 2 for mGluR5) were cut from the SNr and mounted on resin carriers to allow for the collection of ultrathin sections using an ultramicrotome (Ultracut T2, Leica, Nussloch, Germany). The ultrathin sections were collected on single-slot copper grids, stained with lead citrate for 5 minutes to enhance contrast, and examined on a Zeiss EM-10C electron microscope (Thornwood, NY)

Electron micrographs were taken at 10,000x to 31,500x magnifications to characterize the nature of immunoreactive elements in the SNr.

Electrophysiology

Whole-patch clamp recordings were obtained as previously described (Marino *et al.*, 1998; Bradley *et al.*, 2000). 15-18 day old Sprague-Dawley rats were used for all patch clamp studies. After decapitation, brains were rapidly removed and submerged in an ice cold sucrose buffer (in mM: Sucrose, 187; KCL, 3; $MgSO_4$, 1.9; KH_2PO_4, 1.2; Glucose, 20; $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2). Parasagittal, or horizontal slices (300μM thick) were made using a Vibraslicer (WPI). Slices were transferred to a holding chamber containing normal ACSF (in mM: NaCl, 124; KCL, 2.5; $MgSO_4$, 1.3; NaH_2PO_4,1.0; $CaCl_2$, 2.0; Glucose, 20; $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2). In some experiments, 5 μM glutathione, 500

μM pyruvate, and 250 μM kynurenate were included in the sucrose buffer and holding chamber. These additional compounds tended to increase slice viability but did not have any effect on experimental outcome. Therefore, data from these two groups have been pooled. Slices were transferred to the stage of a Hoffman modulation contrast microscope and continuously perfused with room temperature ACSF (~3 mL/min, 23-24°C). Neurons in the substantia nigra pars reticulata were visualized with a 40X water immersion lens. Patch electrodes were pulled from borosilicate glass on a Narashige vertical patch pipette puller and filled with (in mM) potassium gluconate, 140; HEPES, 10: NaCl, 10; EGTA, 0.6; GTP, 0.2; ATP, 2 (pH adjusted to 7.5 with 0.5 N NaOH). Biocytin (0.5%, free base) was added just prior to use. Electrode resistance was 3-7 MΩ. For measurement of synaptically evoked slow EPSPs, bipolar tungsten electrodes were used to apply stimuli to the SNr approximately 100 μm rostral to the recording site. Slow EPSPs were evoked in the presence of 10 μM CNQX, 10 μM D-AP5, 10 μM bicuculline, and 100 μM 2-hydroxy-saclofen. IPSCs were evoked with the stimulation electrode placed within the SNr rostrally or caudally to the recorded cell outside the cerebral peduncle and recorded at a holding potential of -50 mV. 10-20 μM CNQX and 10-20 μM D-AP5 were continuously added to the bath to block excitatory transmission. To study miniature IPSCs (mIPSCs) the 140 mM potassium gluconate in the internal solution were substituted by 140 mM CsCl to reduce postsynaptic mGluR effects and increase currents. Therefore, inward mIPSCs were recorded at a holding potential of -80 mV in the presence 1 μM tetrodotoxin (TTX).

RESULTS

Localization of Group I mGluRs in the SNr. Previous studies have demonstrated the expression of both mGluR1 and mGluR5 in the SNr (Testa *et al.*, 1994, 1998). However, these studies did not address the synaptic localization of these receptors. In order to determine if Group I mGluRs are postsynaptically localized at putative glutamatergic synapses in the SNr, we performed immunocytochemical studies with antibodies selective for mGluR1a and mGluR5.

At the light microscopic level, the SNr exhibited labeling for both mGluR1a (Fig 1) and mGluR5 (Fig 2). In order to determine whether this immunoreactivity represents pre- or postsynaptic staining, we performed immunocytochemical analysis at the electron microscopy level. Both antibodies primarily labeled dendritic processes which formed symmetric and asymmetric synapses with unlabeled terminals (Fig 1,2). While the majority of labeling was postsynaptic, mGluR1a immunoreactivity was also found in small unmeylinated axons and a few axon terminals (Fig 1). In the case of presynaptic labeling for mGluR1a, the immunoreactivity was seen only at symmetric synapses. A few glial processes were also labeled with both antibodies. Most immunoreactive dendrites were tightly surrounded by a large density of striatal-like terminals forming symmetric synapses (Fig 1C-F; 2C-E), an ultrastructural feature typical of SNr GABAergic neurons (Smith and Bolam, 1991). In contrast, SNc dopaminergic neurons are much less innervated (Bolam and Smith, 1990). These data indicate that the majority of immunoreactive elements labeled with the two group I mGluR antibodies belong to SNr GABAergic neurons.

Electrophysiological identification of GABAergic neurons in the SNr. For

electrophysiological analysis of the roles of mGluRs in SNr GABAergic projection neurons, it is critical to differentiate between GABAergic neurons and the smaller population of dopaminergic neurons in this region. Fortunately, these two neuronal types exhibit distinct electrophysiological and morphological features. Therefore, we used electrophysiological criteria that were previously established to distinguish between dopaminergic neurons and GABAergic projection neurons (Nakanishi *et al.*, 1987; Hausser *et al.*, 1995; Richards *et al.*, 1997). GABAergic neurons exhibit a high rate of spontaneous repetitive firing, short duration action potentials (half amplitude duration = 1.7 ± 0.2 msec), little spike accommodation, and a lack of inward rectification (Fig 3). In contrast, dopaminergic neurons display no, or low frequency spontaneous firings, longer duration action potentials (half amplitude duration = 7.0 ± 0.5 msec), strong spike accommodation, and a pronounced inward rectification (Fig 3). Light microscopic examination of biocytin-filled neurons indicated that GABAergic neurons had extensive dendritic arborizations close to the cell body, whereas dopaminergic neurons had sparser dendritic structure (data not shown). All data presented in this study are from electrophysiologically identified GABAergic neurons.

Activation of group I mGluRs depolarizes SNr GABAergic neurons. Previous studies have demonstrated that all three groups of mGluRs are expressed in the SNr (Testa *et al.*, 1994; Testa *et al.*, 1998). We therefore employed maximal concentrations of group-selective mGluR agonists to determine whether activation of these receptors has an effect on membrane properties of SNr GABAergic neurons. In the presence of 0.5 μM tetrodotoxin (TTX), application of the group I mGluR-selective agonist DHPG induces a robust direct depolarization (300 μM DHPG,

16.1 ± 2.6 mV) of SNr neurons which reverses upon drug washout (Fig 4A,C). This depolarization is accompanied by a significant increase in input resistance (pre-drug, 498 ± 70 MΩ; 100 μM DHPG, 619 ± 89 MΩ; $p<0.05$ paired t) (Fig 4B) suggesting that a DHPG-induced decrease in membrane conductance underlies this effect. The concentration-response relationship for DHPG-induced depolarization of SNr GABAergic neurons exhibited an EC_{50} of approximately 40 μM, (Fig 4D) consistent with an effect on group I mGluRs (Schoepp *et al.*, 1994; Gereau and Conn, 1995a). In contrast to this group I mGluR-mediated depolarization, the group II selective agonist LY354740 (Monn *et al.*, 1997; Kingston *et al.*, 1998) and the group III selective agonist L-AP4 (Conn and Pin, 1997) had no significant effect on resting membrane potential (Fig 4A-C). Therefore, we focused on the physiology and pharmacology of the group I mGluR-mediated depolarization.

To determine the effect of group I mGluR activation on action potential firing in SNr GABAergic neurons, we applied the selective group I mGluR agonist DHPG in the absence of TTX. At the beginning of whole cell recording, cells fire spontaneous action potentials (Fig 3) however, within a few minutes, cells tend to hyperpolarize and do not fire spontaneously. Application of 100 μM DHPG induced a robust depolarization, and a large increase in action potential firing (Fig 4E). This DHPG-induced firing is completely blocked by injection of hyperpolarizing current to maintain a -65 mV membrane potential during drug application, and is mimicked by direct depolarization of the cells to the same membrane potential (100 μM DHPG, 3.8 ± 0.3 Hz; direct depolarization 3.2 ± 0.7 Hz, $p>0.05$ students t). These data suggest that the increase in firing was solely due to the depolarization and that mGluR activation did not have other effects on membrane properties of SNr neurons to increase firing frequency.

In other neurons, activation of group I mGluRs has been demonstrated to depolarize the cells by inhibition of a leak potassium conductance (Guerineau *et al.*, 1994), or by an increase in a non-selective cationic conductance (Guerineau *et al.*, 1995; Miller *et al.*, 1995). Our observation that DHPG causes an increase in input resistance suggests that inhibition of leak potassium conductance is the most likely mechanism underlying this effect. Consistent with this, voltage clamp analysis revealed a DHPG-induced inward current underlying the depolarization (Fig5a). Voltage ramps between -40 and -120 mV (20 mV/s) were used to establish a current-voltage relationship of the DHPG induced current. Application of 100 μM DHPG induced a change in the slope of the whole cell current-voltage relationship (Fig 5B). Subtracting the pre-drug I-V trace from the trace in the presence of DHPG reveals a near linear I-V relationship for the DHPG-induced current. which reverses near the calculated potassium equilibrium potential of -103.4 mV (Fig 5C). Binning the data in 10 mV segments and averaging over 5 independent experiments produces a current voltage relationship which is well fit by a straight line ($r=0.995$) and has an interpolated reversal potential of 108.8 ± 8.5 mV, in agreement with the calculated potassium equilibrium potential (Fig 5D). Taken together, these data suggest that the DHPG-induced depolarization of SNr GABAergic neurons is mediated by decreasing a leak potassium conductance.

The DHPG-induced excitation of SNr GABAergic neurons is mediated by mGluR1. Our findings that both mGluR1a and mGluR5 are postsynaptically localized in SNr projection neurons suggests that both of these receptors could be involved in the DHPG-induced depolarization. In order to determine the role each of these receptors plays in this effect we

employed newly available pharmacological tools that distinguish between mGluR1 and mGluR5. CBPG, a partial agonist at mGluR5 which has antagonistic properties at mGluR1 (Yokoi *et al.*, 1996; Mannaioni *et al.*, 1999) failed to induce a depolarization at maximal concentrations (Fig 6A), indicating that the depolarizing effect of DHPG is likely due to activation of mGluR1. Consistent with this, pretreatment with the highly selective, noncompetitive mGluR1 antagonist CPCCOEt (Annoura *et al.*, 1996; Casabona *et al.*, 1997; Litschig *et al.*, 1999), or the highly selective, competitive mGluR1 antagonist LY367385 (Clark et al., 1997) produced a significant reduction in the DHPG-induced depolarization of SNr GABAergic neurons. Pretreatment with MPEP, a highly selective noncompetitive antagonist of mGluR5 had no significant effect at concentrations shown the be effective at blocking mGluR5 in other systems (Gasparini *et al.*, 1999; Bowes *et al.*, 1999) (Fig6).

mGluR1 mediates a slow EPSP in SNr GABAergic neurons. The data presented thus far indicate that mGluR1 mediates direct excitation of SNr projection neurons. The SNr receives a sparse yet important glutamatergic innervation from the STN, and burst firing of the STN is known to play a key role in several neurological disorders including PD (Hollerman and Grace, 1992; Bergman *et al.*, 1994; Hassani *et al.*, 1996) and epilepsy (Deransart *et al.*, 1996; Deransart *et al.*, 1998; Deransart *et al.*, 1999). If activation of glutamatergic afferents to the SNr release sufficient glutamate to activate mGluR1, the resulting excitation of SNr projection neurons could play an important role in these disease states. We tested this hypothesis by recording from SNr GABAergic neurons in the presence of ionotropic glutamate receptor and GABA receptor antagonists. High frequency stimulation (25-50 Hz, 100 μS) of the afferents within the SNr

produced a robust and reliable slow EPSP which reached threshold for action potential firing in 4 of 4 cells (Fig 7). Consistent with mediation by mGluR1, this slow EPSP was completely and reversibly blocked by 100 μM CPCCOEt.

Group I mGluRs decrease inhibitory transmission in the SNr. It was surprising that our immunocytochemical studies revealed light presynaptic staining in the SNr. In some other brain regions, mGluRs can act as heteroreceptors to reduce GABA release and inhibitory synaptic transmission. If activation of group I mGluRs decreases inhibitory transmission in the SNr, this combined with the direct excitatory effects described above, would provide a mechanism whereby group I mGluR activation could exert a powerful excitatory influence on the SNr. We directly tested this hypothesis by recording inhibitory post synaptic currents (IPSCs) in SNr GABAergic projection neurons. IPSCs were evoked by stimulating within the SNr with bipolar stimulation electrodes (0.4-12.0 μA every 30 seconds) and were recorded at a holding potential of -50 mV in the presence of AMPA (CNQX; 10-20 μM) and NMDA (D-AP5; 10-20 μM) receptor antagonists to prevent excitatory synaptic transmission. Bicuculline (10 μM; n=8) abolished evoked IPSCs in all cells tested, confirming that the evoked currents were $GABA_A$ receptor-mediated responses. Short (3 min) bath application of the group I mGluR selective agonist DHPG (100 μM) reduced the amplitude of evoked IPSCs in a reversible manner (Fig. 8A,B). Concentration response analysis revealed that the inhibition of IPSCs by DHPG was concentration dependent with an IC_{50} value of around 50 μM and a maximal effect at a concentration of around 300 μM DHPG (46.0%±9.5, N=4; Figure 8C). This is consistent with the potency of DHPG on group I mGluRs.

Pharmacological studies of the DHPG-induced decrease in inhibitory transmission using subtype selective antagonists where carried out to determine which group I mGluR subtypes mediate this effect. The mGluR5-selective antagonist MPEP (10 μM), had a slight tendency to block the DHPG induced effect, but the response to MPEP did not reach statistical significance (Figure 9B,E; $p > 0.05$). In contrast, the mGluR-selective antagonist, CPCCOEt, induced a significant reduction of the DHPG induced suppression of IPSCs (Figure 9C,E). However, the response to CPCCOEt was only a partial blockade of the response and DHPG still induced a 20.9 ± 4.6 % inhibition of IPSCs in the presence of this antagonist. Since neither antagonist was capable of completely blocking the response when added alone, we also determined the determined effect of a combination of both CPCCOEt and MPEP. The combination of antagonists completely blocked the ability of DHPG to reduce evoked IPSCs, suggesting that both mGluR1 and mGluR5 may participate in regulation of IPSCs in SNr. (Fig 9D,E).

The Group I mGluR-mediated decrease in inhibitory transmission occurs by a presynaptic mechanism. In order to determine whether the group I mGluR-mediated decrease in inhibitory transmission in the SNr is mediated through a presynaptic mechanism, we determined the effect of maximal concentrations of DHPG on frequency and amplitude of spontaneous miniature IPSCs (mIPSCs). All mIPSC recordings were preformed at a holding potential of -80 mV in the presence of CNQX (10-20 μM) and D-AP5 (10-20 μM) to block glutamatergic synaptic currents and 1 μM tetrodotoxin to block activity-dependent release of transmitter. mIPSCs were measured as inward currents with pipettes in which Cl^- (140 mM) was the major anion in the internal solution.

Application of the group I selective agonist DHPG (100 μM) had no significant effect on mIPSC frequency or amplitude (Figure10A,B). This can be seen as a failure to induce a significant shift in the amplitude or inter-event interval cumulative probability plots (Figure 10B). The average mIPSC frequency before drug application was 1.74 ± 0.4 Hz and 1.40 ± 0.4 Hz after application of 100 μM DHPG ($p>0.05$; n=4). The average mIPSC amplitude was 29.3 ± 4.2 pA before and 31.9 ± 3.5 pA after DHPG application ($p>0.05$; n=4). This lack of an effect on mIPSC amplitude and frequency is consistent with a presynaptic site of action for the group I mGluR mediated suppression of synaptic transmission (Parfitt and Madison, 1993; Gereau and Conn, 1995b; Doze *et al.*, 1995; Scanziani *et al.*, 1995; Bradley *et al.*, 2000). To further test this hypothesis, we also determined the effect of DHPG on paired-pulse facilitation of evoked IPSCs. All paired-pulse recordings were made in the presence of CNQX (10-20 μM) and D-AP5 (10-20 μM) with standard internal solution to allow measurement of outward IPSCs. IPSCs were evoked every 30 seconds by paired stimulations of equal strength with a 50 msec interpulse interval. At these intervals paired-pulse facilitation was observed in all recordings (60.2 + 6.3 %, n=11). Only cells that showed an agonist-induced inhibition of the amplitude of the first IPSC of at least 25% were used for analysis. DHPG (30 μM) induced an increase in paired-pulse facilitation (Figure 10C, D) in 5 out of 6 cells examined. In those cells, the average increase in paired pulse facilitation induced by DHPG was 56.1 ± 11.7 % ($p < 0.05$; n=5) over the facilitation seen in the absence of DHPG..

Taken together, these studies suggest that activation of the group I mGluRs mGluR1 and mGluR5 reduce inhibitory transmission in the SNr through a presynaptic mechanism. Furthermore, this decrease in GABAergic inhibition may combine with the direct postsynaptic

excitatory effects of mGluR1 activation to produce a powerful excitation of this crucial basal ganglia output nucleus.

DISCUSSION

The data presented here demonstrate that activation of group I mGluRs produces an excitation of the SNr. Both mGluR1 and mGluR5 are found at postsynaptic sites in the SNr, and mGluR1a is sparsely localized at presynaptic terminals in this region. Activation of group I mGluRs produces an excitation of SNr output neurons by two distinct mechanisms. Activation of postsynaptically localized group I mGluRs on SNr GABAergic neurons produces a robust depolarization that induces a marked increase in action potential firing. The depolarization is accompanied by a decrease in membrane conductance, and the underlying current has a reversal potential consistent with mediation by inhibition of a leak potassium channel. Furthermore, this effect is due to selective activation of mGluR1, and can be produced by synaptically released glutamate. Activation of group I mGluRs also induces a decrease in inhibitory transmission in the SNr. This effect is mediated by both mGluR1 and mGluR5, and occurs through a presynaptic mechanism.

Since the glutamatergic projection from the STN constitutes a large percentage of the excitatory terminals on SNr GABAergic neurons, it is likely that the primary source of glutamate acting on group I mGluRs is released from STN afferents. However, several other regions including the pedunculopontine nucleus (Charara *et al.*, 1996) and the nucleus raphe (Corvaja *et al.*, 1993) provide a sparse projection accounting for a small percentage of asymmetric terminals in the SNr. Therefore, group I mGluRs may also modulate these inputs. Interestingly, although we find both mGluR1 and mGluR5 postsynaptically localized in SNr neurons, our pharmacological studies demonstrate that activation of mGluR1 is solely responsible for the group I-mediated depolarization. This is of interest since both mGluR1 and mGluR5 couple to

phosphoinositide hydrolysis, and are capable of inducing depolarization of other neuronal populations (see Conn and Pin, 1997; Anwyl, 1999 for reviews). Thus, specificity of function must be produced by differences in subsynaptic localization or some other functional segregation of these receptors. It should be noted that while mGluR1 plays the predominate role in mediating the group I mGluR-induced depolarization in the SNr, mGluR5 may play important physiological roles regulating cell properties that were not measured in the present study. For example, group I mGluRs are known to modulate NMDA receptor currents in a variety of brain regions, and it is possible that mGluR5 is involved in a similar modulation in SNr. Future studies on the role of mGluR5 in these cells may provide important insight into the distinct functional roles of closely related receptor subtypes within a single neuronal population.

In addition to the postsynaptic labeling of neurons in the SNr for both group I mGluR subtypes, we also detected light presynaptic staining. Consistent with this, we find that activation of the presynaptic group I mGluRs decreases inhibitory transmission. This finding is of particular interest for understanding the role the STN plays in modulation of the SNr. The indirect pathway is composed of striatal projections through the globus pallidus and the STN which constitute a large percentage of the excitatory terminals on SNr GABAergic neurons (Smith *et al.*, 1998). While the glutamatergic input to the SNr is sparse, it plays a critical role in basal ganglia function as evidenced by the pronounced clinical effects of STN lesions in PD (Guridi and Obeso, 1997). The STN also plays a key role in pathological activity of the SNr. Transition of STN neurons from single spike activity to burst-firing mode, and resultant over excitation of the SNr has been implicated in the pathophysiology associated with PD (Hollerman and Grace, 1992; Bergman *et al.*, 1994; Hassani *et al.*, 1996) as well as some forms of epilepsy

(Deransart *et al.*, 1996; 1998; 1999). Furthermore, STN neurons exhibit extremely high firing rates, and can typically exceed 25-50 Hz during burst-firing mode (Hollerman and Grace, 1992; Bergman *et al.*, 1994; Wichmann *et al.*, 1994; Beurrier *et al.*, 1999; Bevan and Wilson, 1999). The robust excitatory effects of mGluR1 activation described here could play an important role in the powerful control exerted by the relatively sparse glutamatergic input to this nucleus from the STN.

Our current findings add to a growing body of literature suggesting that group I mGluRs play important roles in regulating function of the basal ganglia motor circuit. For instance, mGluR5 mRNA is heavily expressed in the striatum, and is also present at lower levels in the STN and the pallidal complex (Testa *et al.*, 1994; Testa *et al.*, 1995; Kerner *et al.*, 1997; Tallaksen-Greene *et al.*, 1998). While the levels of mGluR1 mRNA are more limited, this receptor is also found throughout the basal ganglia (Testa *et al.*, 1994; Kerner *et al.*, 1997; Tallaksen-Greene *et al.*, 1998). A number of studies suggest that agonists of group I mGluRs may act at several levels to increase the net activity of projection neurons in the basal ganglia output nuclei. For instance, activation of group I mGluRs potentiates NMDA receptor currents in striatal neurons (Colwell and Levine, 1994; Pisani *et al.*, 1997). Furthermore, behavioral studies combined with studies of changes in 2-deoxyglucose uptake and fos immunoreactivity suggest that injection of group I mGluR agonists in the striatum induces a selective activation of the indirect pathway from the striatum and thereby increases activity of the output nuclei (Kaatz and Albin, 1995; Kearney *et al.*, 1997). In addition, recent physiological studies suggest that activation of group I mGluRs has profound excitatory effects on STN projection neurons (Abbott *et al.*, 1997; Awad and Conn, 1999). These previous studies, taken together with the present data

suggest that group I mGluRs function at 3 major sites to increase overall output of the basal ganglia motor circuit.

One of the obvious implications of the finding that group I mGluRs are effective at modulating the basal ganglia output nuclei is that these receptors could provide a novel target for the development of pharmacological treatments of basal ganglia disorders. Based on our current studies, and the previous studies discussed above, it is possible that a group I mGluR-selective antagonist could act at multiple sites within the basal ganglia motor circuit to decrease activity through the indirect pathway and decrease excitatory drive to the output nuclei. Since PD is associated with an increase in activity of the indirect pathway and output nuclei, these combined actions could ameliorate the symptoms of hypokinetic movement disorders such as PD.

It is also important to note that the STN sends excitatory projections to the dopaminergic neurons of the substantia nigra pars compacta (SNc). Based on this, it has been postulated that any manipulation that decreases STN-induced excitation of SNc neurons could also reduce excitotoxic damage to the SNc and thereby slow progression of PD. Interestingly, recent studies reveal that activation of group I mGluRs also depolarizes dopaminergic SNc neurons and participates in the synaptic excitation of these cells (Fiorillo and Williams, 1998). This raises the exciting possibility that group I mGluR antagonists might provide both symptomatic relief and slow the dopaminergic neuronal death in PD patients. However, the response to group I mGluR activation in SNc neurons is more complex than that described here for SNr GABAergic neurons and includes a rapid transient hyperpolarization followed by a more lasting depolarization. Thus, the overall effect of group I mGluR action on SNc neurons will likely depend on the temporal context of their activation.

In addition to the potential role of group I mGluRs in the treatment of PD, it is possible that group I mGluR agonists could provide a therapeutic benefit to patients suffering from hyperkinetic movement disorders such as Huntington's disease, or Tourette's syndrome. Current models of the changes in basal ganglia function in these disorders suggest that activity of the basal ganglia indirect pathway and output nuclei decreased in a manner opposite to the changes seen in PD (Wichmann and DeLong, 1997, 1998). In future studies, it will be important to test these hypothesis further using behavioral studies in animal models of these disorders.

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FIGURE LEGENDS

Figure 1. The group I mGluR1a subtype is localized at postsynaptic sites within the SNr. (A) Low power light micrograph of mGluR1a immunostaining in the SNc and SNr. (B) High power light micrograph of mGluR1a immunoreactive processes in the SNr. Lightly labeled neuronal cell bodies are indicated by asterisks. (C) Low power electron micrograph of mGluR1a immunoreactive dendrites (Den) in SNr. Note that the immunoreactivity is mostly found in dendritic processes but also occurs in small, unmeylinated axons (Ax) and a few axon terminals (Te). (D-E) High power electron micrographs of mGluR1a-immunoreactive dendrites that form asymmetric (arrowheads) and symmetric (arrow) synapses with unlabeled terminals. (F) High power electron micrograph showing an mGluR1a-immunoreactive terminal in contact with a small, labeled dendrite. Note also the presence of an immunoreactive glial process (Gl) surrounding an unlabeled terminal. Scale bars: A: 500μm, B: 50 μm, C: 1 μm, D-F: 0.5 μm.

Figure 2. The group I mGluR5 subtype is localized at postsynaptic sites within the SNr. (A) Low power light micrograph of mGluR5 immunostaining on the SNc and SNr. (B) High power light micrograph of mGluR5-immunoreactive processes in the SNr. Labeled cell bodies are indicated by asterisks. (C) Low power electron micrograph of mGluR5-immunoreactive dendrites (Den) in the SNr. Note that the mGluR5 immunoreactivity is confined to dendritic processes. (D-E) High power electron micrograph of mGluR5-immunoreactive dendrites and spines (Sp) that form asymmetric synapses (arrowheads) with unlabeled terminals. Note the presence of an immunoreactive glial process (Gl). Scale bars: A: 500 μm, B: 50 μm, C: 1μm, D-E: 0.5 μm.

Figure 3. Demonstration of the identification of SNr GABAergic neurons. (A) Response of a GABAergic (left) and dopaminergic (right) neuron to depolarizing and hyperpolarizing current injections. Note the pronounced spike frequency adaptation and inward rectification exhibited by the dopaminergic cell which is absent in the GABAergic cell. (B) Examples of spike activity from resting cells. GABAergic neurons (left) fire at high frequency, while dopaminergic neurons (right) exhibit lower frequency or no spontaneous activity. (C) comparison of single action potentials from a GABAergic (left) and dopaminergic (right) neuron. All data presented in this study are from electrophysiologically identified GABAergic neurons.

Figure 4. DHPG induces a group I mGluR-mediated depolarization of SNr neurons. (A) 100 μM DHPG induces a depolarization and (B) concominant increase in input resistance in SNr GABAergic neurons. Maximal concentrations of the group II-selective agonist LY354740, and the group III-selective agonist L-AP4 are without effect. (C) Mean ± SEM of data from 5 cells demonstrating that at maximal concentrations, only the group I agonist DHPG induces a depolarization. (D) Concentration-response relationship of the DHPG-induced depolarization. (E) The effect of DHPG applied in the absence of TTX to demonstrate the robust increase in firing produced by activation of group I mGluRs.

Figure 5. Analysis of mGluR-mediated current in SNr GABAergic neurons. (A) Application of 100 μM DHPG induces an inward shift in holding current which reverses on drug washout. (B) This inward shift is evident in the whole cell current-voltage relation determined by applying voltage ramps from -40 to -120 mV. (C) Subtraction reveals a linear current which is inward at

normal resting potentials, and reverses near the predicted potassium equilibrium potential. (D) Mean ± SEM of data from 5 cells. The interpolated reversal potential is -108.8 ± 8.5 mV which compares favorably with the calculated potassium equilibrium potential of -103.4 mV.

Figure 6. The group I mGluR-induced depolarization is mediated by mGluR1. (A) representative traces demonstrating that the DHPG-induced depolarization of SNr GABAergic neurons is not mimicked by the mGluR5 selective agonist CBPG. Furthermore, preincubation with the highly selective noncompetitive mGluR1 antagonist CPCCOEt fully blocks the DHPG-induced depolarization, while the mGluR5 selective antagonist MPEP is without effect. (B) Mean ± SEM of data from 5 cells per condition demonstrating the selective antagonism of the group I mGluR-mediated depolarization of SNr projection neurons by the mGluR1 selective antagonist CPCCOEt, or the highly selective competitive mGluR1 antagonist LY367385, (*$p<0.01$ students t).

Figure 7. mGluR1 mediates a slow EPSP in SNr GABAergic neurons. High frequency stimulation of afferents in the SNr in the presence of ionotropic glutamate receptor and GABA receptor antagonists elicits a slow EPSP which exceeds action potential threshold and induces firing. This EPSP is fully and reversibly blocked by the selective mGluR1 antagonist CPCCOEt. Traces are representative of results obtained in four independent experiments.

Figure 8. Activation of group I mGluRs decreases inhibitory transmission in the SNr. (A) Representative traces of evoked IPSCs before (pre-drug), during (DHPG), and after washout of a

brief bath application of 100 μM DHPG. (B) Average time course of the effect of 100 μM DHPG , each point represents the mean (± SEM) of data from 5 cells. (C) Dose-response relationship of DHPG-induced suppression of IPSCs. The inhibition of IPSCs shows an IC_{50} of around 50 μM and is maximal at 300 μM. Each point represents the mean (± SEM) of 3-4 experiments.

Figure 9. The group I mGluR-mediated decrease in inhibitory transmission involves both mGluR1 and mGluR5. (A-D) Traces of evoked IPSCs before (control), during, and after (washout) bath application of DHPG alone (A) or in the presence of selective antagonists (B-D). Selective antagonists include 10 μM MPEP (mGluR5 selective, (B)) and 100 μM CPCCOEt (mGluR1 selective, (C)) and the combination of both (D). (E) Bar graph showing the average effect of selective antagonists on the DHPG-induced inhibition of IPSCs. Each bar represents the mean (± SEM) of data collected from 8 cells. (*$p<0.05$; **$p<0.01$).

Figure 10. Figure 5. Inhibition of IPSCs induced by the activation of group I mGluRs is mediated by a presynaptic mechanism. (A) Examples of mIPSC traces before (pre-drug) and during application of 100 μM DHPG. (B) Amplitude histograms of mIPSCs before (left) and during application of 100 μM DHPG (right). (C) Cumulative probability plots showing a lack of effect of DHPG on mIPSC amplitude (left) and inter-event interval (right). Data shown are pooled from 4 experiments. (D) Traces of paired-pulse experiments before (pre-drug) and during application of 30 μM DHPG. On the right an overlay of the pre-drug trace (straight line) and a trace during application of DHPG scaled to the amplitude of the first IPSC (dashed line) is shown. DHPG increases the ratio of paired-pulse facilitation in 5 out of 6 cells.

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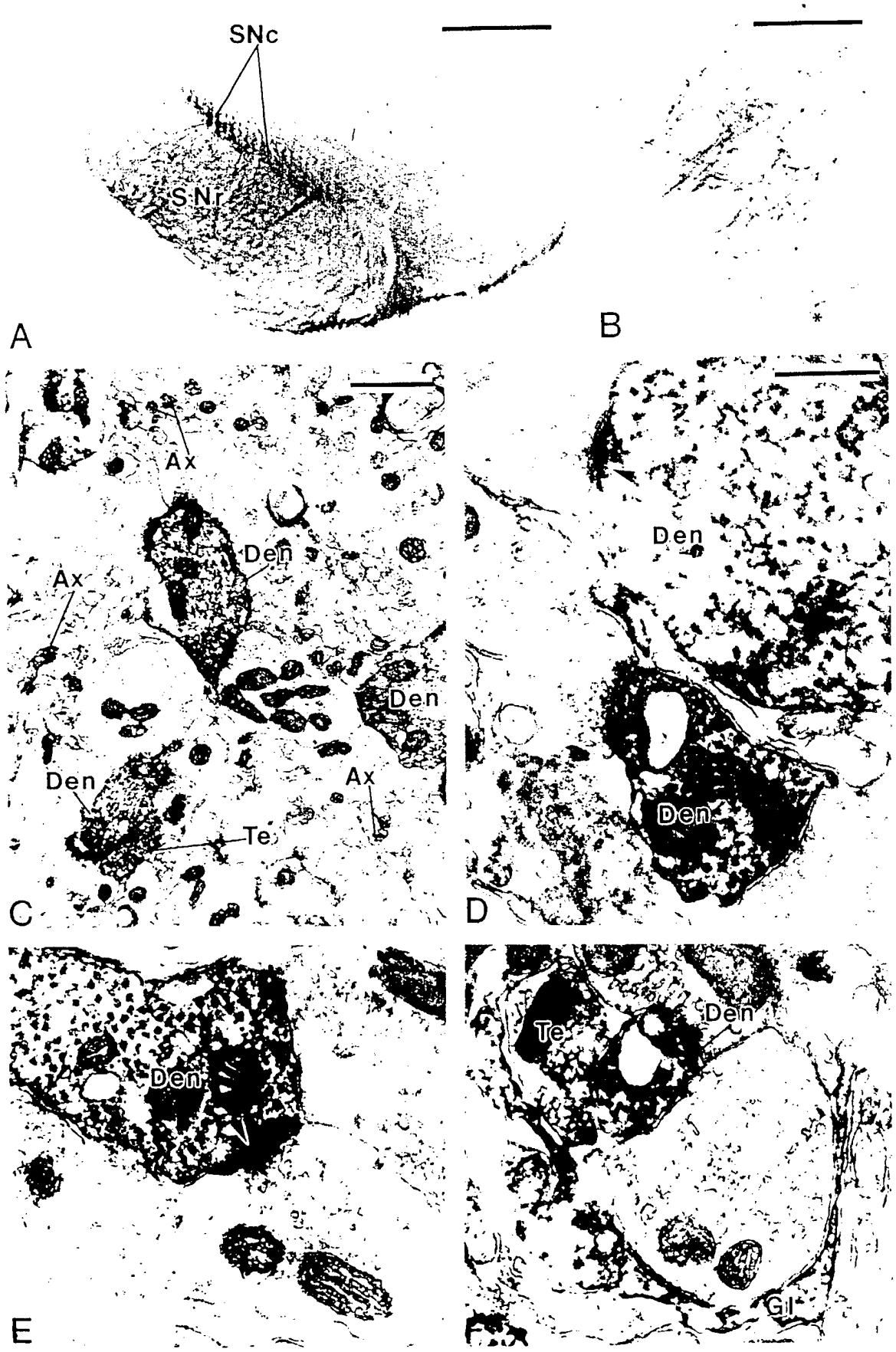
Figure 1



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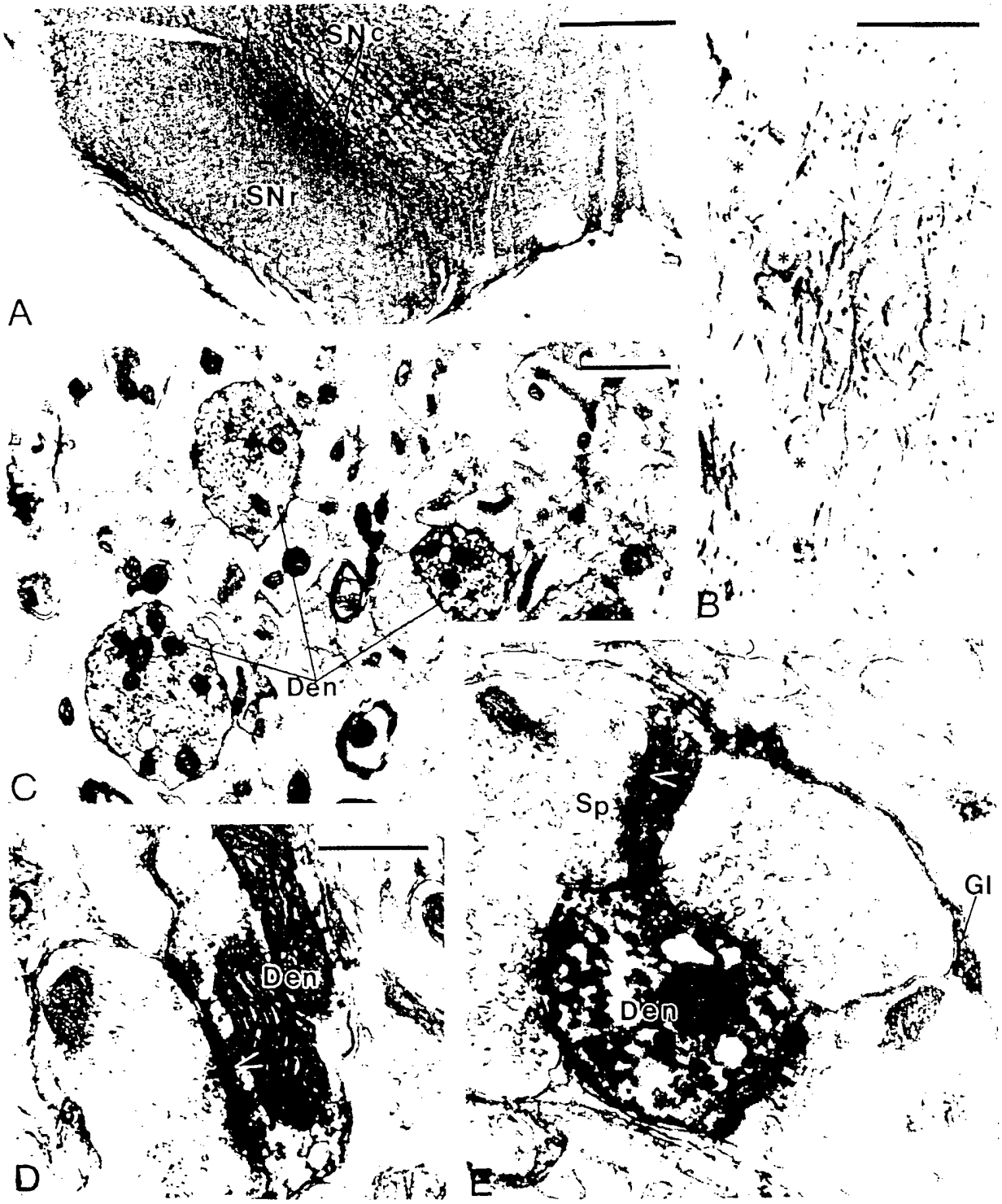

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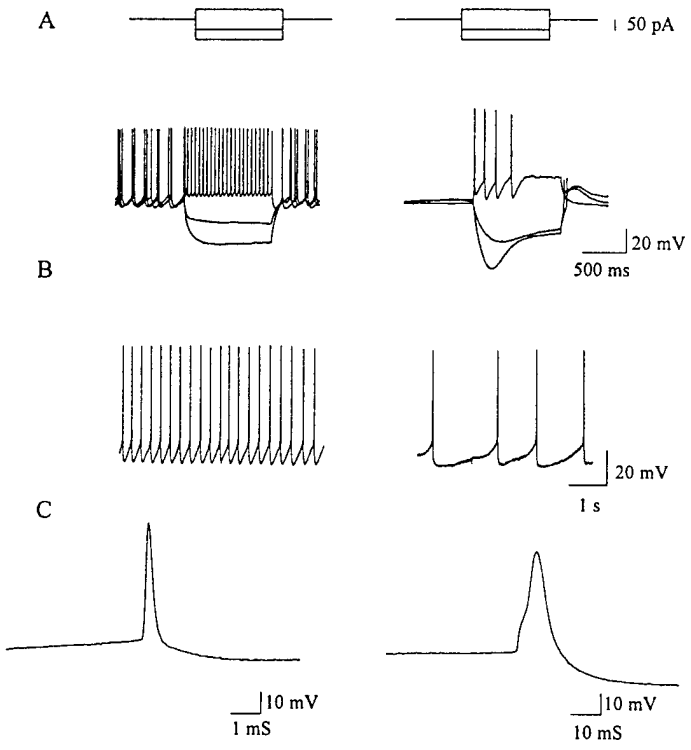

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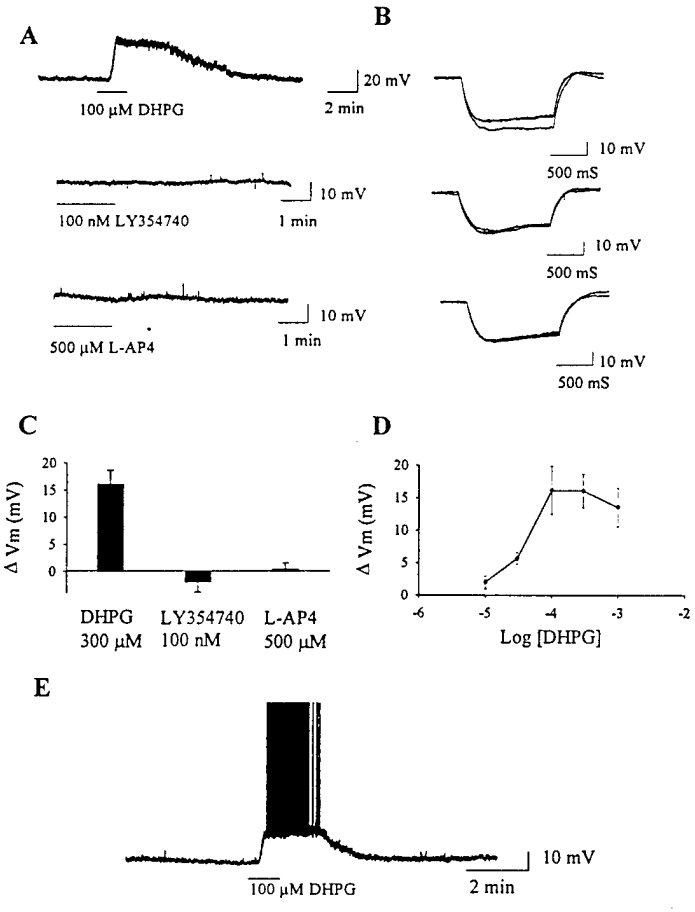

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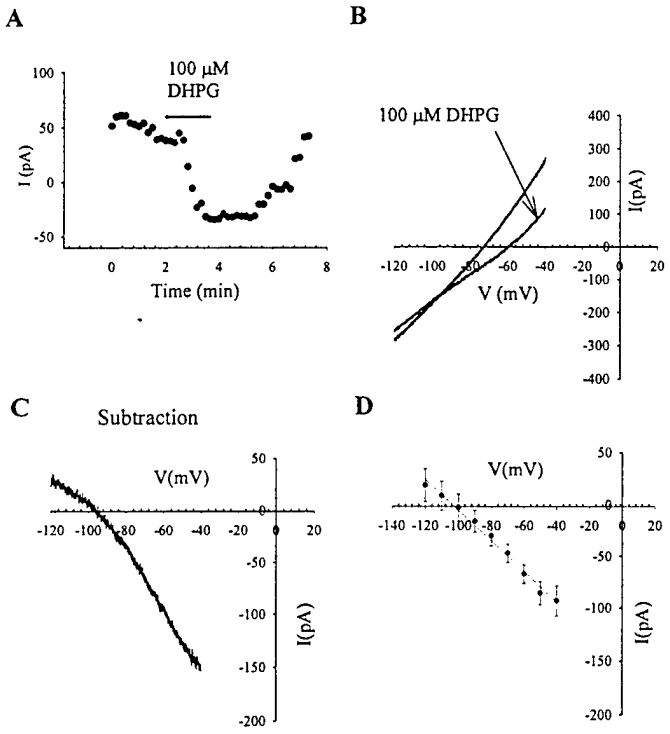

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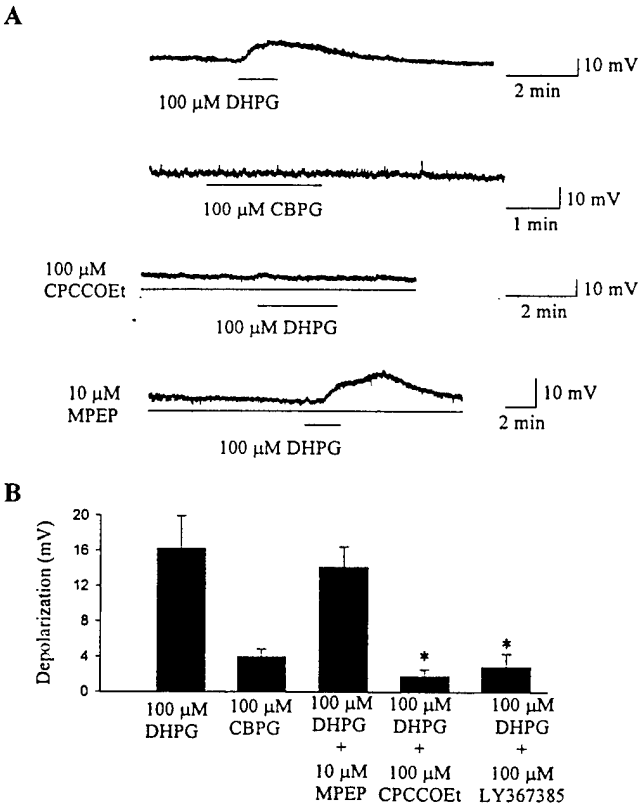

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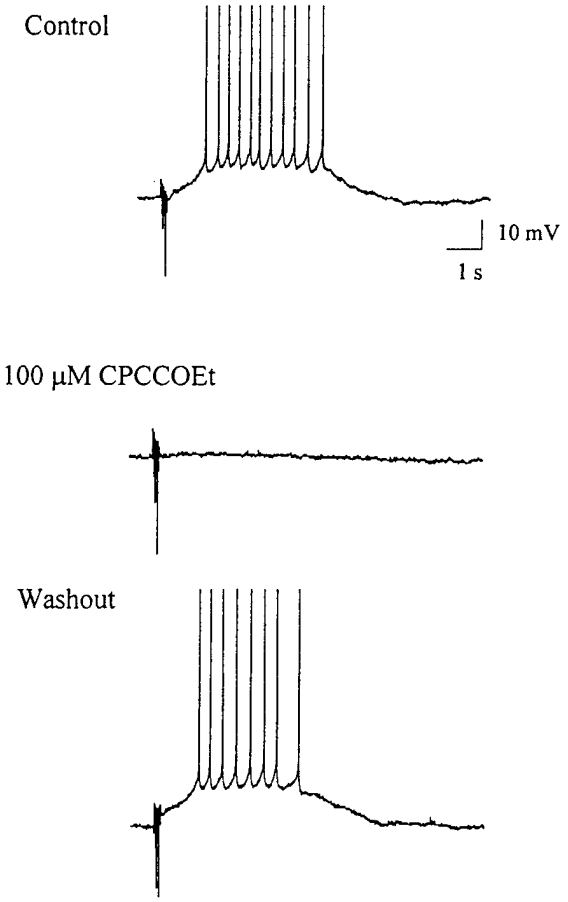

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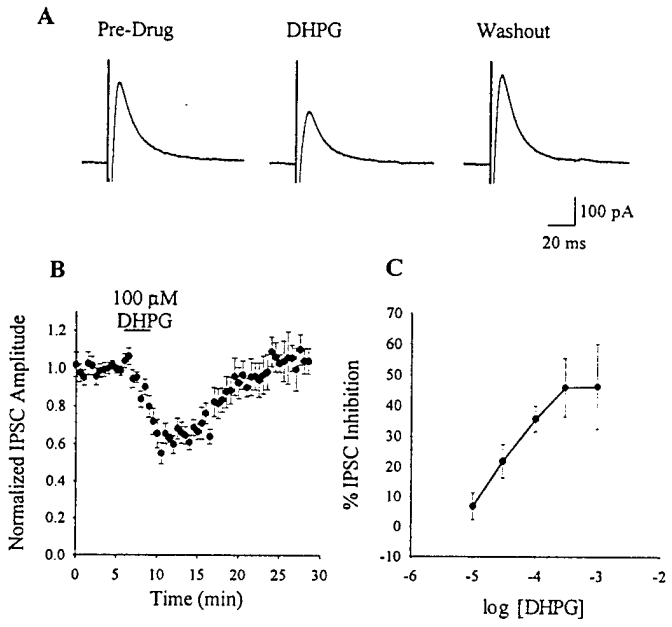

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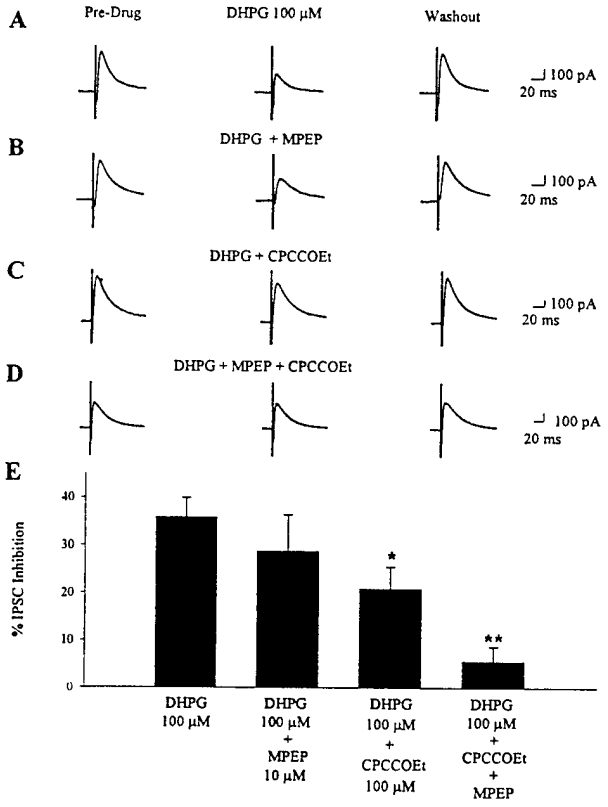

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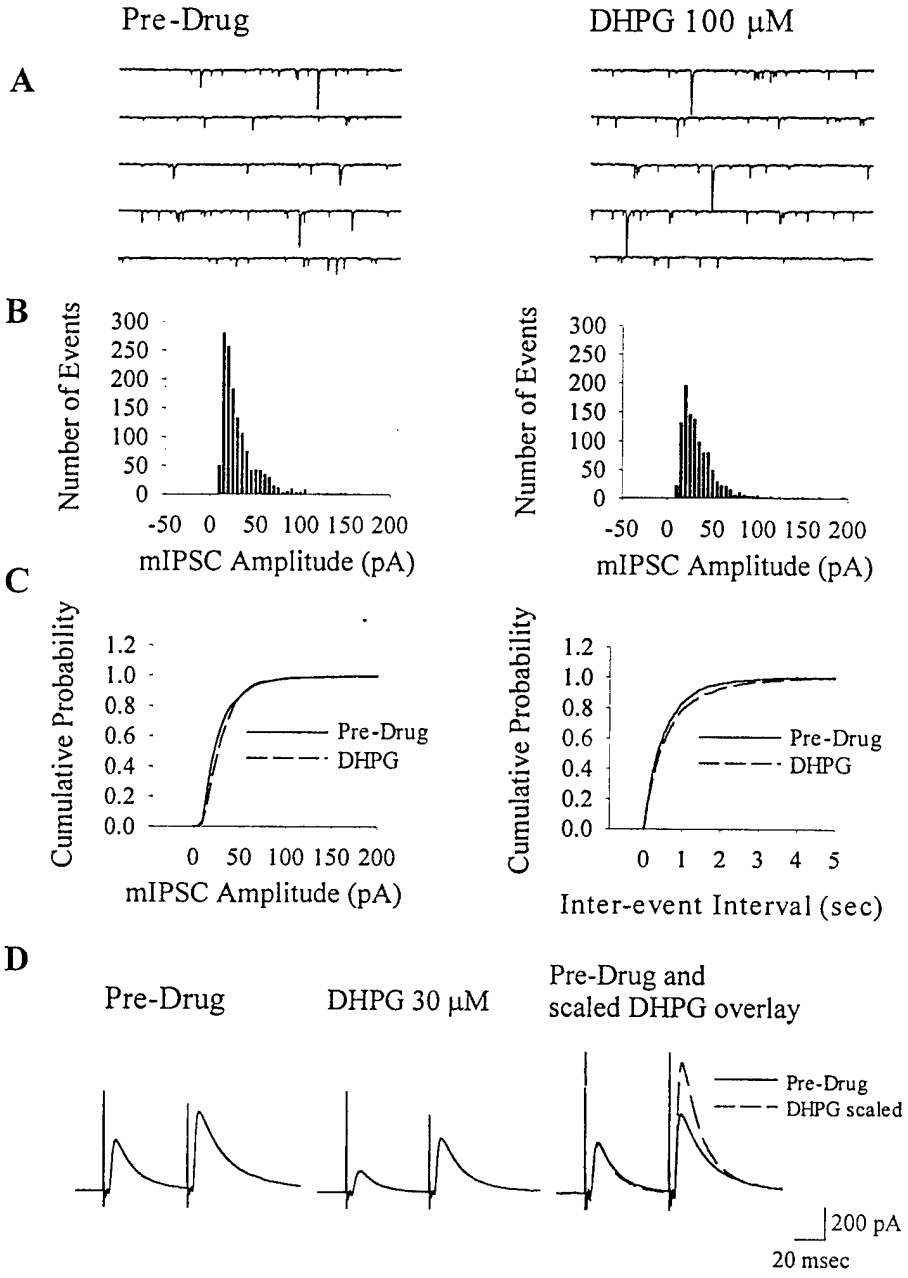

Distribution and roles of metabotropic glutamate receptors in the basal ganglia motor circuit: implications for treatment of PD and related disorders

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Abstract:

The basal ganglia are a set of interconnected subcortical structures that play a critical role in motor control. The basal ganglia are thought to control movements by a delicate balance of transmission through two basal ganglia circuits that connect the input and output nuclei: the direct pathway and the indirect pathway. The basal ganglia are also involved in a number of movement disorders. Most notably, the primary pathophysiological change that gives rise to the motor symptoms of Parkinson's Disease is the loss of dopaminergic neurons of the substantia nigra pars compacta that are involved in modulating function of the striatum and other basal ganglia structures. This ultimately results in an increase in activity of the indirect pathway relative to the direct pathway and the hallmark Parkinson's Disease symptoms of rigidity, bradykinesia and akinesia. A great deal of effort has been dedicated to finding treatments for this disease. The current pharmacotherapies are aimed at replacing the missing dopamine, while the current surgical treatments are aimed at reducing transmission through the indirect pathway. Dopamine replacement therapy has proven to be helpful but is associated with severe side effects that limit treatment and a loss of efficacy with progression of the disease. Recently developed surgical therapies have been highly effective but are highly invasive, expensive, and assessable to a small minority of patients. For these reasons, new effort has been dedicated to finding pharmacological treatment options that will be effective in reducing transmission through the indirect pathway. Members of the metabotropic glutamate receptor (mGluR) family have emerged as interesting and promising targets for such a treatment. This review will explore the most recent advances in the understanding of mGluR localization and function in the basal ganglia motor circuit and the implications of those findings for the potential therapeutic role of

mGluR-targeted compounds for Parkinson's Disease.

Key Words:

Basal ganglia

Parkinson's Disease

Metabotropic glutamate receptors

Therapeutics

Presynaptic

Abbreviations:

mGluR-metabotropic glutamate receptor

BG-basal ganglia

PD-Parkinson's Disease

GABA-gamma-aminobutyric acid

GPi-Globus Pallidus, internal segment

EPN-entopenduncular nucleus

SNr -substantia nigra, pars reticulata

SNc- substantia nigra, pars compacta

GPe-globus pallidus, external segment

STN-subthalamic nucleus

TS-Tourette's Syndrome

L-DOPA-levodopa

ACPD-(+/-)-1-aminocyclopentane-*trans*-1,3-dicarboxcylic acid

DHPG-(RS)-3,5-dihydroxyphenylglycine

DCG-IV-(2S, 2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine

MPEP-methylphenylethynylpyridine

CPCCOEt-7-hydroxyiminocyclopropan-[b]chromen-1a-carboxylic acid ethyl ester

EPSP-excitatory postsynaptic potential

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1. Introduction

The basal ganglia (BG) is a collective term used to refer to a group of interconnected subcortical nuclei that provide a feedback loop to many different areas in the cortex and descending influences to brainstem motor regions. One of the most prominent roles of the BG is regulation of motor function. The most striking evidence for such a role is the movement disorder, Parkinson's disease (PD). The clinical syndrome that occurs in PD patients is characterized by a disabling motor impairment that includes tremor, rigidity, and bradykinesia. A large number of basic and clinical studies reveal that the primary pathophysiological change giving rise to the symptoms of PD is a loss of substantia nigra dopaminergic neurons that are involved in modulating function of the striatum and other basal ganglia structures. In order to understand the dysfunction of PD, and related movement disorders, it is essential to gain a clear understanding of the normal circuitry and function of the BG.

2. Circuitry of the Basal Ganglia Motor System.

The left panel of figure 1 provides a schematic diagram of the major aspects of BG circuitry. While this diagram is highly oversimplified, it provides a useful framework for examining basal ganglia function. The main excitatory input to the BG is from the motor cortex. The cortex sends excitatory projections to the striatum, the major input nucleus of the BG. Corticostriatal fibers synapse onto striatal GABAergic projection neurons termed medium spiny neurons for their distinct morphology. These projection neurons can be subdivided into two functional classes. Approximately half of the medium spiny neurons project directly to the BG output nuclei, the globus pallidus internal segment (GPi; the entopeduncular nucleus (EPN) in rats) and the substantia nigra pars reticulata (SNr; for see Smith, A. D. et al. (1990). This is called the direct pathway and provides direct inhibitory control over the BG output

nuclei. The inhibitory control over the GABAergic GPi/SNr output cells provided by the direct pathway ultimately leads to a disinhibition of thalamocortical cells. The other half of the striatal projection neurons participate in a multi-synaptic relay referred to as the indirect pathway. GABAergic medium spiny neurons giving rise to the indirect pathway project to and inhibit GABAergic cells of the globus pallidus external segment (GPe). GPe cells normally exert tonic inhibitory control over glutamatergic cells of the subthalamic nucleus (STN), but activation of the GABAergic striatopallidal pathway leads to a disinhibition of the STN. This allows for excitatory transmission between the STN and the BG output nuclei, the GPi/SNr, thus inhibiting thalamocortical cells (Bergman, H. et al., 1990; DeLong, M. R., 1990).

The direct and indirect pathways of the BG act as a fine tuning mechanism in movement control (Alexander, G. et al., 1986). The balance of transmission through the direct and indirect pathways is tightly regulated by a major modulatory projection from dopaminergic neurons in the substantia nigra pars compacta (SNc). This dopamine input to the striatum regulates the direct and indirect pathways differentially due to the presence of different postsynaptic dopamine receptors on the two populations of medium spiny neurons. D1 receptors are primarily expressed on medium spiny neurons that project directly to SNr, while D2 receptors are primarily expressed on by the medium spiny neurons which constitute the indirect pathway (Gerfen, C. R. et al., 1990). Because of this differential expression the release of dopamine in the striatum has a net excitatory effect on the direct pathway, and an inhibitory influence on the indirect pathway.

As mentioned above, the primary pathological change giving rise to the motor symptoms of Parkinson's Disease is the selective death of dopaminergic neurons in the SNc. The loss of this important modulatory input, results in a decrease in activity through the direct pathway and an increase

in activity through the indirect pathway (Albin, R. L. et al., 1989; Wichmann, T. et al., 1997). These changes lead to increased inhibition of thalamocortical neurons, which is believed to underlie the hallmark symptoms of the disease: rigidity, bradykinesia and akinesia. The right panel of figure 1 right schematically illustrates the activity changes in basal ganglia-thalamocortical circuitry that are thought to occur in PD. Interestingly, evidence suggests that opposite changes in transmission through the direct and indirect pathways may occur in some other movement disorders. For instance, Tourette's syndrome (TS) is a relatively common neuropsychiatric disorder that is characterized by motor and phonic tics that can include sudden repetitive movements, gestures, or utterances. According to current models, TS is associated with an increase in striatal dopamine, or in the dopamine sensitivity of striatal neurons, that has effects that are opposite to those seen in PD patients. Thus, the increase in dopaminergic transmission is thought to lead to an increase in activity through the direct pathway and a corresponding decrease in activity through the indirect pathway (Albin, R. L. et al., 1989; Leckman, J. F. et al., 1997). Huntington's disease is another hyperkinetic disorder that is thought to be due to a selective loss of striatal spiny neurons that give rise to the indirect pathway (Albin, R. L. et al., 1990; Reiner, A. et al., 1988). Again, this should lead to a decrease in activity through the indirect pathway relative to the direct pathway (Albin, R. L. et al., 1990; Reiner, A. et al., 1988).

3. Therapeutic restoration of balance of activity through the direct and indirect pathways.

Treatment of PD has traditionally utilized strategies for replacing lost dopamine and thereby restoring the critical dopaminergic modulation of basal ganglia function. Levodopa (L-DOPA), the immediate precursor of dopamine, was the first highly effective treatment for PD and remains the most effective drug for treating the motor manifestations of PD (see Poewe, W. H. et al. (1997) for extensive

review). However, while effective early in treatment for a majority of patients, L-DOPA and other dopamine replacement therapies have a number of serious shortcomings. Within 5 years of beginning treatment, most patients begin to experience motor fluctuations and the efficacy of L-DOPA becomes unpredictable. In addition, patients begin to develop serious side effects that often limit therapy (Poewe, W. H. et al., 1986; Poewe, W. H. et al., 1997). Because of this, there has been a major focus on developing novel treatment strategies that are aimed at acting downstream of the lost dopaminergic neurons to restore balance the direct and indirect pathways. This effort has led to development of highly effective surgical treatments, such as pallidotomy (Baron, M. S. et al., 1996; Laitinen, L. V. et al., 1992) or high frequency stimulation of the subthalamic nucleus (Limousin, P. et al., 1995), that are aimed reducing activity through the indirect pathway. These treatments have been successful in ameliorating parkinsonian symptoms in a subset of patients. Unfortunately, these surgical approaches are highly invasive, extremely expensive and remain reserved for patients that can no longer be helped by dopamine replacement therapy.

4. Metabotropic glutamate receptors provide novel therapeutic targets for treatment of movement disorders.

In recent years, a novel family of receptors for the amino acid transmitter glutamate have been characterized that couple to effector systems through GTP-binding proteins. These receptors, referred to as metabotropic glutamate receptors (mGluRs), are widely distributed throughout the central nervous system where they play important roles in regulating cell excitability and synaptic transmission (see Conn, P. J. et al. (1997) for extensive review). One of the primary functions of mGluRs is a role as presynaptic receptors involved in reducing transmission at glutamatergic synapses. The mGluRs also

serve as heteroreceptors involved in reducing GABA release at inhibitory synapses. Finally, postsynaptically localized mGluRs often play an important role in regulating neuronal excitability and in regulating currents through ionotropic glutamate receptor channels. If mGluRs play these roles in BG, then members of this receptor family may provide exciting new targets for drugs that restore the balance between the direct and indirect pathway without the problems associated with dopamimetic therapy and could be useful for relieving the symptoms of PD and related movement disorders. Interestingly, recent studies suggest that members of this receptor family are differentially distributed in several BG nuclei where they play an important role in regulating neuronal signaling.

To date, eight mGluR subtypes have been cloned from mammalian brain. These subtypes are classified into three major groups based on sequence homologies, coupling to second messenger systems and pharmacological profiles. Group I mGluRs, which include mGluR1 and mGluR5, couple primarily to G_q and increases in phosphoinositide hydrolysis. Group II mGluRs (mGluR2 and mGluR3) and group III mGluRs (mGluR4, 6, 7 and 8) couple to G_i/G_o and inhibition of adenylyl cyclase (for review see Conn, P. J. et al., (1997) and Anwyl, R. (1999)). This review will provide a brief survey of studies of mGluR localization and function in the basal ganglia motor circuit and the potential relevance of these findings for treatment of PD and other movement disorders.

5. mGluRs modulate excitatory input to the BG

The major excitatory drive to the BG, which originates in the cortex and terminates in the striatum (corticostriatal pathway), is modulated presynaptically by group II and III mGluRs. Activation of presynaptic group III mGluRs on corticostriatal results in an inhibition of glutamatergic transmission (Calabresi, P. et al., 1992; East, S. J. et al., 1995; Lombardi, G. et al., 1993; Lovinger, D. M. et al., 1995;

Pisani, A. et al., 1997a). This effect is likely mediated by the mGluR4 or 7 subtypes, as they have been identified via electron microscopic immunocytochemistry at asymmetric (excitatory) synapses in the striatum (Fig. 2; Bradley, S. R. et al., 1999b; Bradley, S. R. et al., 1999a; Kosinski, C. M. et al., 1999). In addition, group II specific agonists have been shown to inhibit transmission at this synapse (Lovinger, D. M. et al., 1995). In other brain regions, group II and group III mGluRs act as presynaptic autoreceptors at glutamatergic synapse. These studies suggest that the mGluRs may play a similar role in modulating corticostriatal excitation (Testa, C. M. et al., 1998). The group I mGluRs, mGluR1 and mGluR5, have been identified in striatal medium spiny neurons (Fig. 2; Kerner, J. A. et al., 1997; Testa, C. M. et al., 1995; Testa, C. M. et al., 1998). Activation of group I mGluRs potentiates NMDA receptor currents in striatal neurons (Colwell, C. S. et al., 1994; Morari, M. et al., 1994; Pisani, A. et al., 1997b).

It is difficult to make clear predictions regarding the net effect of agonists and antagonists acting at mGluRs in striatum on transmission through the direct and indirect pathways. This would depend on whether these receptors selectively regulate specific populations of the striatal projection neurons. However, behavioral studies with injection of group I mGluR agonists combined with measurements of c-fos expression in the STN suggest that activation of group I mGluRs may selectively increase transmission through the indirect pathway (Kaatz, K. W. et al., 1995; Kearney, J. A. et al., 1997; Kearney, J. A. et al., 1998; Sacaan, A. I. et al., 1991; Sacaan, A. I. et al., 1992). If so, antagonists of the group I mGluRs could provide a therapeutic benefit to PD patients by selectively reducing activity through the indirect pathway. Likewise, agonists of these receptors may provide a therapeutic benefit for patients suffering from Tourette's syndrome or Huntington's disease.

6. mGluRs modulate dopaminergic transmission from the SNc to the striatum

The dopaminergic pathway from the substantia nigra pars compacta to the striatum is also under modulatory control of all three mGluR groups. All three groups of mGluRs have been shown to depress transmission at glutamatergic synapses in the SNc (Wigmore, M. A. et al., 1998). These effects are likely presynaptic and suggest that mGluRs are acting as autoreceptors on glutamatergic afferents from the STN, pedunculopontine nucleus and cortex. Since glutamate release in the SNc is thought to contribute to the degeneration of the SNc neurons observed in PD, it is possible that an mGluR agonist may have the potential to slow the progression of the disease. However, future studies are necessary to determine the role of STN over-activity in contributing to the dopaminergic cell death and to identify the exact mGluR subtypes involved at this synapse.

In addition to presynaptic inhibitory actions, postsynaptic mGluRs play an excitatory role in the SNc. Direct activation of group I mGluRs depolarizes dopaminergic cells (Meltzer, L. T. et al., 1997; Mercuri, N. B. et al., 1993; Shen, K. Z. et al., 1997). Interestingly brief application of group I mGluR agonists induces a biphasic response. The initial inhibitory phase of this response is mediated by group I receptors was only observed at low agonist concentration and very short application time. This inhibitory effect has been shown to be mediated by the activation of a calcium dependent potassium conductance (Fiorillo, C. D. et al., 1998). Anatomical studies confirm that mGluR1 mRNA and protein is indeed localized postsynaptically in dopaminergic SNc neurons (Fig. 2; Kosinski, C. M. et al., 1998; Testa, C. M. et al., 1994).

mGluRs also play a presynaptic regulatory role at the nigrostriatal synapse. ACPD (a non-selective mGluR agonist) as well as DHPG (a group I specific agonist) and DCG-IV (a group II selective agonist) all facilitate dopamine release from nigrostriatal terminals (Bruton, R. K. et al., 1999; Ohno, M. et al., 1995; Verma, A. et al., 1998). This suggests that cortical glutamate release, in addition to directly

exciting striatal output neurons, will actually increase dopamine release and facilitate the dopaminergic-mediated increase in direct pathway activity. This might suggest that groups I mGluR agonists could have therapeutic benefit in Parkinson's Disease increasing neurotransmitter release from the surviving dopaminergic terminals. However, the competing effects in the STN and SNr that are discussed below would likely counteract this.

7. mGluRs modulate transmission through the direct pathway

Approximately half of the medium spiny neurons in the striatum project directly to the output nuclei of the BG, the GPi and the SNr, constituting the direct pathway. Anatomical studies indicate that the mGluR7 subtype is presynaptically localized to symmetric (inhibitory) synapses in the SNr (Fig. 2; Kosinski, C. M. et al., 1999). These afferents originate in the striatum. Physiological evidence from our laboratory indicates that activation of group I and III mGluRs results in disinhibition of nigral output neurons by decreasing GABAergic inhibitory transmission (Wittmann, M. et al., 1999). This resulting increase in the activity of the SNr projection neurons could increase the inhibitory drive from the GABAergic output nuclei onto thalamocortical neurons. This suggests that group III mGluR-selective agonists could decrease inhibition of the BG output through the direct pathway and may provide a novel target for treating hyperkinetic disorders such as Huntington's Disease and Tourette's syndrome.

8. mGluRs modulate transmission through the indirect pathway

a. Striatopallidal synapse:

The striatal enkephalinergic medium spiny neurons that give rise to the indirect pathway project to the GPe in primates (Anderson, K. D. et al., 1990; Beckstead, R. M. et al., 1985). The GPe is referred

to simply as the globus pallidus (GP) in rodents where most of the mGluR studies have been performed. This is the first synapse in the indirect pathway. While no functional studies of mGluRs at this synapse have been performed, anatomical studies have demonstrated the existence of mGluR receptors at both sides of the striatopallidal synapse. The group III mGluRs, mGluR4 and 7 have been localized to presynaptic striatopallidal terminals using both confocal and electron microscopy (Fig. 2; Bradley, S. R. et al., 1999b; Bradley, S. R. et al., 1999a; Kosinski, C. M. et al., 1998). GP neurons express mRNA and protein for mGluR1 and mGluR5 (Fig. 2; Testa, C. M. et al., 1994). Electron microscopic studies have shown postsynaptic localization of mGluR1 (Testa, C. M. et al., 1998) and mGluR7 (Bradley, S. R. et al., 1999b; Kosinski, C. M. et al., 1998) in rat and mGluR5 in primate (Fig. 2; Hanson, J. E. et al., 1999). The anatomical distribution of mGluRs at the striatopallidal synapse has some interesting, hypothetical functional roles. While the primary neurotransmitter released from striatopallidal terminals is GABA, the GP also receives a small glutamatergic input from the STN (Plenz, D. et al., 1998; Shink, E. et al., 1995). Activation of the STN could result in activation of presynaptic mGluRs on striatopallidal terminals, thus inhibiting their release of GABA. This would ultimately result in an increase in GP activity that inhibits STN activity. Additionally, STN excitatory input could directly activate pallidosubthalamic neurons via postsynaptic mGluRs on GP neurons that would, in turn, inhibit STN neurons. This negative feedback loop likely acts as an STN regulator in a normally functioning BG. However, when the indirect pathway is overactive, as it is in PD, the small glutamatergic input to the GP may not be enough to keep STN activity in check.

The localization of mGluRs in the GP has some interesting therapeutic implications. Of particular note, if an increase in activity of striatopallidal neurons is indeed important for the motor dysfunction associated with PD, agonists of mGluR4 and/or mGluR7 could reduce transmission at this

synapse an provide a therapeutic benefit. However, while there is wide agreement that increased activity of the STN is critical for PD, there is less agreement on the relative role of GP (Hassani, O. K. et al., 1996; Levy, R. et al., 1997). Thus, it is not yet certain that reducing inhibition of GP would dramatically reduce the overactivity of STN neurons in parkinsonian animals. Another issue to consider is that if group III mGluRs are also present on terminals of direct striatal projections to the output nuclei, this would counteract the beneficial effect of group III agonists in the GP. Consistent with this possibility, immunocytochemistry studies reveal that mGluR7 is evenly distributed at striatopallidal and striatonigral synapses (Kosinski, C. M. et al., 1999). However, mGluR4 appears to be more abundant in striatopallidal synapses than in striatonigral synapses (Bradley, S. R. et al., 1999a). Thus, it is conceivable that selective agonists of mGluR4 could selectively reduce transmission through the indirect pathway while having less effect on the direct pathway. In the future, it will be important to perform detailed physiological and behavioral studies to further evaluate the potential therapeutic value of group III mGluR agonists in the GP.

b. The pallidosubthalamic synapse:

In contrast to the striatopallidal synapse, less is known about the anatomical distribution of mGluR subtypes at the pallidosubthalamic synapse. However, recent anatomical and functional studies do suggest an important role of mGluRs in the STN. *In situ* hybridization studies have shown that the pallidosubthalamic neurons express mGluR1 and 5 mRNA, and subthalamic neurons express mGluR1, 2, 3 and 5 mRNA (Testa, C. M. et al., 1994). Recently, mGluR1 and 5 (group I) have been localized to dendrites of subthalamic neurons (Fig. 2; Awad, H. et al., 1999a; Awad, H. et al., 1999b). These receptor proteins were found to be postsynaptic at both symmetric (inhibitory, most likely GABAergic

pallidal fibers) and asymmetric (excitatory) synapses.

Physiological studies have demonstrated that activation of group I mGluRs induces a robust depolarization of STN neurons (Awad, H. et al., 1999a; Awad, H. et al., 1999b). Interestingly, this depolarization is mediated primarily by the group I subtype, mGluR5. The mGluR5 specific antagonist, MPEP, but not the mGluR1 specific antagonist, CPCCOEt, blocked the depolarization. A functional role for the postsynaptic mGluR1 receptor proteins has yet to be determined. In addition to directly depolarizing STN neurons, group I activation also increases the frequency of STN burst firing (Awad, H. et al., 1999a; Awad, H. et al., 1999b; Beurrier, C. et al., 1999). This is particularly interesting, as the switch from single spike firing to a burst firing mode is one of the characteristics of parkinsonian states in animal models (Bergman, H. et al., 1994; Hassani, O. K. et al., 1996; Hollerman, J. R. et al., 1992) and parkinsonian patients (Benazzouz, A. et al., 1996; Rodriguez, M. C. et al., 1997). If this switch does play a role in the neuropathology of the disease, then group I antagonists may potentially be therapeutic targets.

c. *The subthalamonigral synapse:*

As described above, the over-activity of the glutamatergic subthalamic nucleus projection to the BG output nuclei results in an inhibition of thalamocortical neurons. An extremely effective surgical treatment for PD, high frequency stimulation of the STN (Limousin, P. et al., 1995), is designed to shut down or diminish excitatory input to the EPN/SNr from the STN. In rodents, the primary BG output nucleus is the SNr. Also, to date, the only study of mGluRs in the entopeduncular nucleus (homologous to the primate GPi) has been an *in situ* hybridization study, and the results closely parallel *in situ* findings in the SNr (Testa, C. M. et al., 1994). Therefore, we will focus on studies of the STN-SNr

synapse.

Neurons in the STN express mRNA for mGluR1, 2, 3, and 5 (Testa, C. M. et al., 1994). Electron microscopic immunocytochemical studies have identified mGluR2/3 (Bradley, S. R. et al., 1999c) and 7 (Fig. 2; Bradley, S. R. et al., 1999b; Kosinski, C. M. et al., 1998) at presynaptic terminals in the SNr that are making asymmetric (excitatory) synapses onto SNr dendrites. These excitatory terminals presumably originate in the STN. GABAergic projection neurons of the SNr express mRNA for mGluR1, 3 and 5 (Testa, C. M. et al., 1994). Immunocytochemical studies have demonstrated that mGluR1 and 5 are postsynaptically localized at both asymmetric and symmetric synapses in these neurons (Fig. 2; Marino, M. J. et al., 1999a; Testa, C. M. et al., 1998). The localization of mGluR subtypes postsynaptic to inhibitory inputs is an intriguing finding. Future studies will be required to elucidate the role of these postsynaptic receptors at non-glutamatergic synapses.

Several recent physiological studies in our laboratory have shed significant light on the role of mGluRs at the subthalamonigral synapse. Both group II (Fig. 3) and group III receptors inhibit glutamatergic transmission at this synapse (Bradley, S. R. et al., 2000; Marino, M. J. et al., 1999a). This is consistent with anatomical data demonstrating the presence of mGluR2/3 and 7 presynaptically localized at this synapse (Fig. 2; Bradley, S. R. et al., 2000). Activation of group I mGluRs produces a robust direct depolarization of SNr GABAergic neurons (Marino, M. J. et al., 1999b). This effect appears to be mediated by mGluR1, as the mGluR1-selective antagonist CPCCOEt, but not the mGluR5-selective antagonist, MPEP, block it. Therefore, in contrast to the group I mediated depolarization in STN that was attributable to mGluR5, the analogous effect in SNr seems solely attributable to mGluR1. Moreover, stimulation of glutamatergic afferents to the SNr at frequencies consistent with the normal firing rate of STN neurons induces an mGluR-mediated slow EPSP that is

blocked by CPCCOEt (Marino, M. J. et al., 1999b). These data suggest that mGluR1 may play an important role in tonic regulation of BG output.

Our findings that mGluR2/3 is presynaptically localized on presumed STN terminals in SNr and that activation of group II mGluRs inhibits excitatory transmission in the SNr is of particular interest to potential alternative therapeutic approaches for PD. A mGluR agonist may be useful in reducing transmission at the STN-SNr synapse. This receptor subtype is a particularly interesting target due to its restricted localization in the BG. The only other cells in the BG found to express mGluR2 are the striatal cholinergic interneurons (Testa, C. M. et al., 1994). Therefore, agonists selective for mGluR2 may be useful in alleviating the over-activity of the indirect pathway without triggering many undesirable side effects. Consistent with this hypothesis, recent behavioral studies have demonstrated that the systemic injection of a the highly selective group II agonist, LY 354740, decreases haloperidol-induced muscle rigidity (Konieczny, J. et al., 1998) and catalepsy (Fig. 3; Bradley, S. R. et al., 2000) in a rat model of PD. Furthermore, injection of another group II mGluR agonist, DCG-IV, into the SNr has a similar antiparkinsonian effect (Dawson, L. et al., 2000), suggesting that the effect of group II agonist is mediated by reducing transmission in the SNr.

9. Summary of the potential mGluR targeted pharmacotherapies

All of these studies taken together, demonstrate that multiple mGluR subtypes may be promising potential targets for pharmacotherapies for PD. Antagonists of group I mGluRs may be useful in reducing transmission through the indirect pathway. A group I mGluR antagonist could have antiparkinsonian effects by actions at several different sites in the basal ganglia motor circuit including the striatum, STN, SNr neurons. A potential drawback to a non-selective group I mGluR antagonist for

treatment of PD is that it would also block the group I mGluR-mediated increase in dopamine release from the SNc neurons. However, since the other sites of action of a group I antagonist are downstream from dopamine neurons, this action may not significantly alter the therapeutic effect of a group I antagonist. A group III mGluR agonist may also be useful in ameliorating the symptoms of PD. Again a non-selective group III mGluR agonist may not be ideal, because it would reduce GABAergic transmission at both the striatonigral and striatopallidal synapses. This would reduce transmission through both pathways, thus yielding no net effect. However, mGluR4 appears to be selectively expressed in striatopallidal synapses and may provide a more specific target for attempting to reduce transmission through the indirect pathway. Finally, group II mGluR agonist may provide the most promising opportunity to date for designing an mGluR-mediated therapy for PD. This type of drug would decrease transmission through the indirect pathway by decreasing glutamatergic transmission at the STN-SNr synapse. The restricted expression pattern of mGluR2 in the basal ganglia makes this receptor a particularly interesting target. Additionally, the behavioral studies performed to date using a group II agonist have demonstrated promising results.

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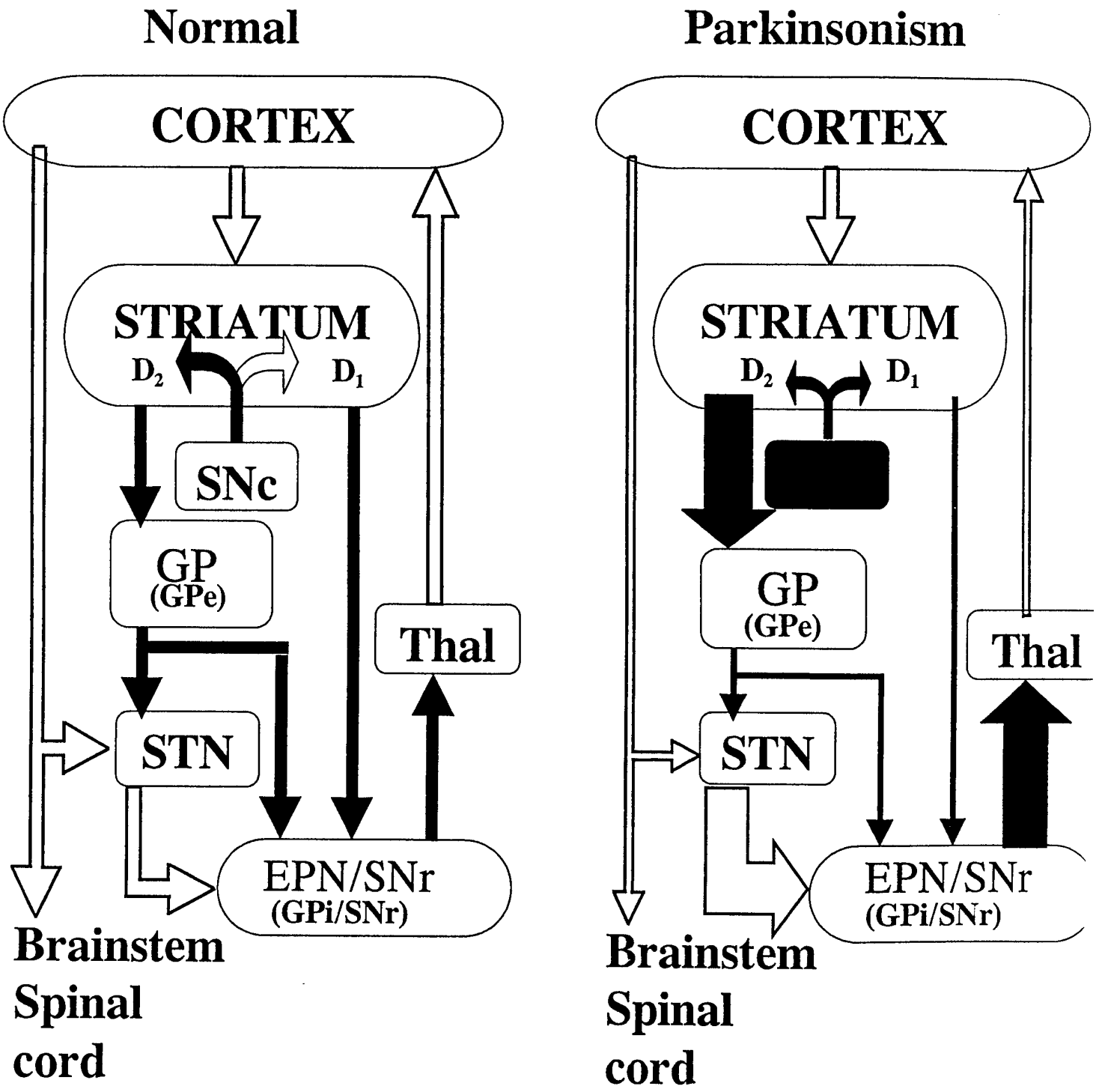

Figure 1
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Figure 1: A schematic representation of basal ganglia circuitry. The left panel represents normal transmission through the normal basal ganglia. The right panel represents the imbalanced transmission in the Parkinsonian basal ganglia. The solid (black) arrows represent inhibitory projections and the open arrow represent excitatory projections. The thickness of the arrows represents the relative activity of that particular projection.

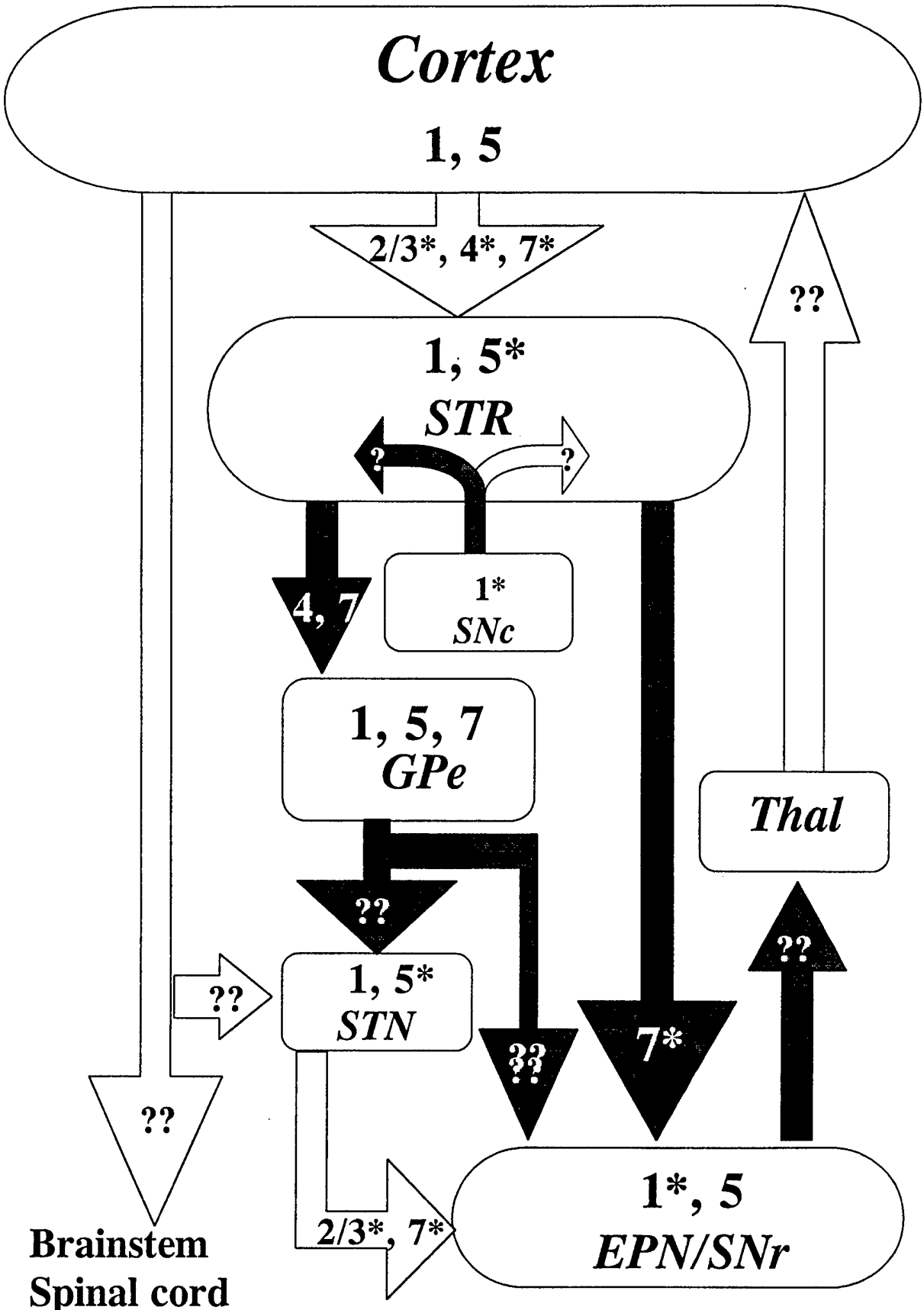

Figure 2
Rouse et al.

Figure 2: A schematic representation showing the confirmed immunoreactivity of mGluR proteins in the basal ganglia motor circuit. Numbers in the nuclei boxes represent postsynaptic localization of those subtypes in that nucleus, whereas numbers in the arrows represent presynaptic localization of those subtypes in that projection. An asterisk indicates that a physiological role has been experimentally defined for that subtype in the location. Solid (gray) arrows represent inhibitory projections and the open arrow represent excitatory projections.

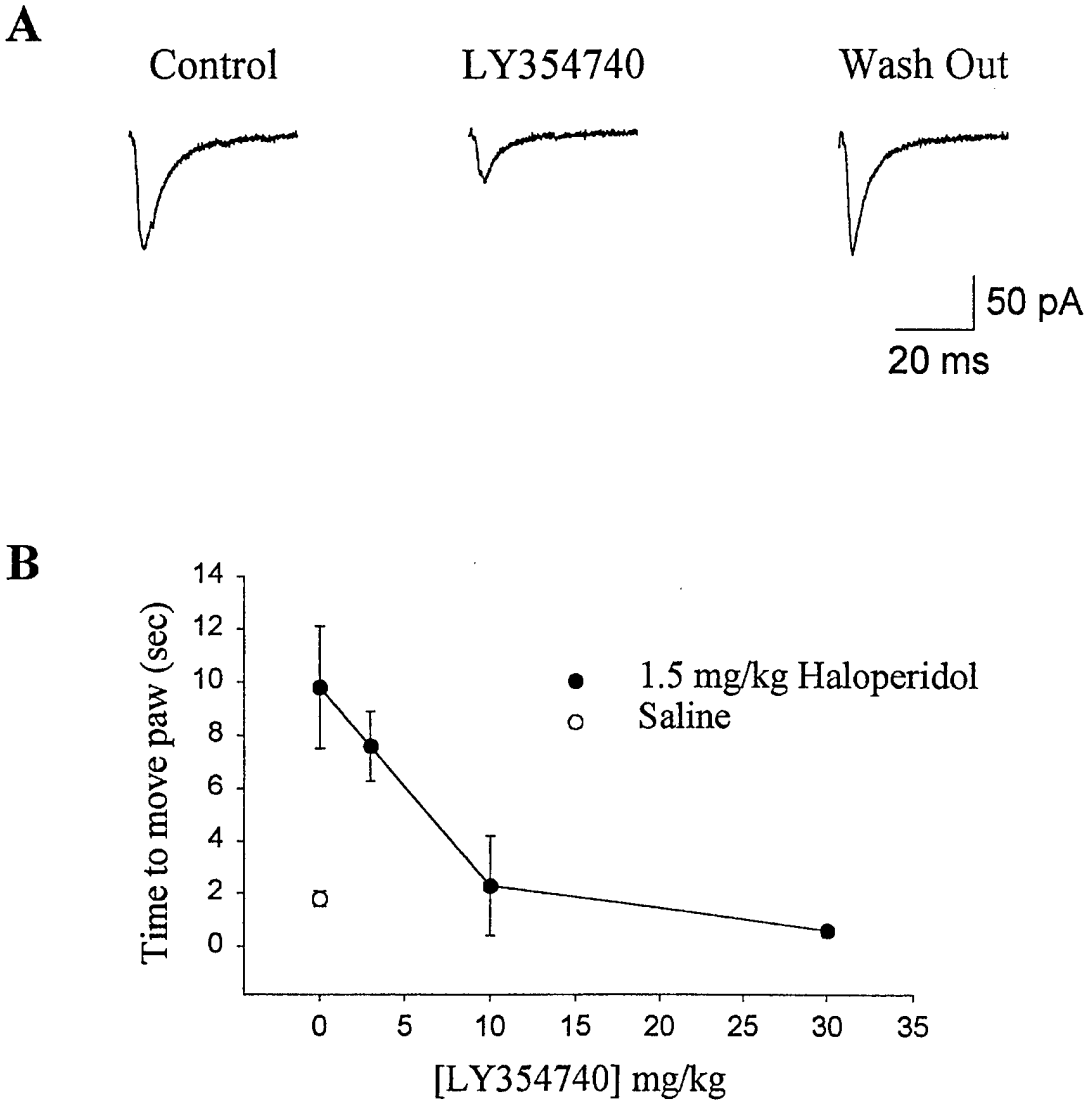

Figure 3
Rouse et al.

Figure 3- Group II mGluRs modulate transmission at the STN-SNr synapse.

(A) Evoked EPSCs before (control), during (LY354740), and after (wash out) brief local application of LY354740. Applications of LY354740 dramatically reduce EPSCs, and this effect is reversible. The effect of LY354740 is mimicked by other group II mGluR-selective agonists and blocked by group II mGluR-selective antagonists (Bradley et al., 2000)

(B) Activation of group II mGluRs reverses catalepsy in an animal model of Parkinson's disease. Degree of haloperidol-induced catalepsy was measured as latency to first paw movement when the animal was placed on a vertical grid. Haloperidol (1.5 mg/kg I.P.) induces a pronounced catalepsy which was reversed in a dose dependent manner by LY354740 (3-30 mg/kg I.P.) (* $p<0.05$). LY354740 alone had no effect (data not shown). Data shown represent mean SEM of data collected from 8 animals.

LOCALIZATION AND PHYSIOLOGICAL ROLES OF METABOTROPIC GLUTAMATE RECEPTORS IN THE INDIRECT PATHWAY

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INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by disabling motor impairments including tremor, rigidity, and bradykinesia. The primary pathological change giving rise to the symptoms of Parkinson's disease is loss of dopaminergic neurons in the substantia nigra pars compacta that modulate the function of neurons in the striatum and other nuclei in the basal ganglia (BG) motor circuit (Fig. 1). Currently, the most effective pharmacological agents for treatment of PD include levodopa (L-DOPA), the immediate precursor of dopamine, and other drugs that replace the lost dopaminergic modulation of BG function [1]. Unfortunately, dopamine replacement therapy ultimately fails in most patients due to loss of efficacy with progression of the disease and severe motor and psychiatric side effects [2]. Because of this, a great deal of effort has been focused on developing new approaches for treatment of PD.

The primary input nucleus of the basal ganglia is the striatum (caudate, putamen, and nucleus accumbens), which receives dense innervation from the cortex and subcortical structures. The primary output nuclei of the basal ganglia are the substantia nigra pars reticulata (SNr) and the entopeduncular nucleus (EPN) which send GABAergic projections to the thalamus. The current model of cortical information flow through the basal ganglia states that the striatum projects to these output nuclei both directly, and indirectly through the globus pallidus and subthalamic nucleus (STN)[3,4]. The direct pathway provides a GABAergic inhibition of the SNr/EPN, while the projection to globus pallidus relieves a GABAergic inhibition of STN, resulting in a glutamatergic excitation of SNr/EPN. A delicate balance between the inhibition of the output nuclei by the direct pathway, and excitation by the indirect pathway is believed to be crucial for control of movement, and any imbalance in this system underlies the pathophysiology of movement disorders.

Recent studies reveal that loss of nigro-striatal dopamine neurons results in a series

Figure 1. A model of how the Parkinson's-related loss of dopamine neurons in the SNc impacts information flow throught the basal ganglia. Note the increase of glutamatergic transmission at the STN-SNr synapse. Inhibitory connections are depicted by black arrows, excitatory transmission depicted by white arrows. Figure modified from reference 3.

of neurophysiological changes that lead to over activity of the indirect pathway, resulting in a pathological excitation of the STN. Increased activity of STN neurons leads to an increase in glutamate release at STN synapses onto GABAergic projection neurons in the output nuclei. This glutamate-mediated over excitation of BG output ultimately produces the motor impairment characteristic of PD [5]. Discovery of the pivotal role of increased activity in the indirect pathway in PD has led to a major focus on surgical approaches for treatment. For instance, lesions or high frequency stimulation of the STN provides a therapeutic benefit to PD patients[6]. In addition, pallidotomy, a surgical lesion of the GP, produces similar therapeutic effects by reversing the impact of increased activity of STN neurons[7,8]. Development of these highly effective neurosurgical approaches provides a major advance in our understanding of the pathophysiology of PD. However, surgical approaches are not widely available to Parkinson's patients. Due to their invasive nature, high cost, and considerable expertise required, such treatment is reserved for patients that are refractory to dopamimetic therapy.

An alternative to surgical approaches to reducing the increased excitation of basal ganglia output nuclei in PD patients would be to employ pharmacological agents that counteract the effects of over activation of the STN neurons by reducing transmission through the indirect pathway. One approach would be to target metabotropic glutamate receptors (mGluRs). Eight mGluR subtypes have been cloned (designated mGluR1-mGluR8) from mammalian brain. These mGluRs are classified into three major groups based on sequence homologies, coupling to second messenger systems, and selectivities for various agonists. Group I mGluRs, which include mGluR1 and mGluR5, couple primarily to increases in phosphoinositide hydrolysis. Group II mGluRs (mGluR2 and mGluR3), and group III mGluRs (mGluR4, 6, 7,and 8) couple to inhibition of adenylyl cyclase. The mGluRs are widely distributed throughout the central nervous system and play important roles in regulating cell excitability and synaptic transmission (for review see [9,10]). One of the primary functions of the

mGluRs is a role as presynaptic receptors involved in reducing transmission at glutamatergic synapses. The mGluRs also serve as heteroreceptors involved in reducing GABA release at inhibitory synapses. Finally, postsynaptically localized mGluRs often play an important role in regulating neuronal excitability and in regulating currents through ionotropic glutamate receptors. If mGluRs play these roles in basal ganglia, particularly in the indirect pathway, members of this receptor family may provide an exciting new target for drugs that could be useful for the treatment of PD, as well as other disorders of BG function. In this chapter we will describe our current understanding of mGluR distribution and function in the indirect pathway. Unless otherwise noted, all results presented are from studies of rat basal ganglia.

THE STRIATO-PALLIDAL SYNAPSE

The indirect pathway arises from the striatal enkephalinergic medium aspiny neurons [11,12]. These GABAergic neurons project to cells in the GP, forming the first synapses in the indirect pathway. Striatal neurons express mRNA for group I, II and III mGluRs [13]. Of these, the group III mGluRs, mGluR4 and mGluR7, have been localized to presynaptic striato-pallidal terminals using both confocal and electron microscopy [14-16]. Neurons in the GP express mRNA for mGluR1 and 5 [13], and are immunoreactive for mGluR7 [15,16]. Postsynaptic localization has been demonstrated for mGluR1 [17], and mGluR7 [15,16]. In addition, mGluR5 has been localized to postsynaptic sites at primate striato-pallidal synapses[18].

To date there have been no studies on the function of the mGluRs at the striato-pallidal synapse. However, the receptor localization raises some interesting possibilities. While the primary input to the pallidum is GABAergic, there is some sparse glutamatergic input from the STN [19]. Therefore, activation of the STN could directly excite pallidal neurons by actions on postsynaptic ionotropic and metabotropic glutamate receptors, and disinhibit pallidal neurons by actions on presynaptic mGluRs modulating GABA release. The resulting excitation of the GP would in turn inhibit the STN. This inhibitory feedback loop may play a role in regulating the balance of activity through the indirect pathway under normal conditions. However, in the case of PD, the sparse glutamatergic input may be insufficient to maintain this feedback control. The potential therapeutic value of restoring balance at this site will be determined by future studies on the role of group III mGluRs in modulating transmission at this synapse.

THE PALLIDO-SUBTHALAMIC SYNAPSE

In contrast to the striato-pallidal synapse, relatively little is known about the distribution of mGluRs at the pallido-subthalamic synapse. The projection neurons of the GP express mRNA for mGluR1 and 5, and the glutamatergic projection neurons of the STN express mGluR1, 2, 3, and 5 mRNA[13] Recently, the group I mGluRs have been postsynaptically localized to dendrites of STN neurons at both symmetric and asymmetric synapses [20,21]

Activation of group I mGluRs induces a robust depolarization of STN neurons [20,21]. Interestingly, this depolarization is blocked by the mGluR5-selective antagonist MPEP, but not by the mGluR1-selective antagonist CPCCOEt, indicating that only one of the group I mGluRs (mGluR5) localized at this synapse mediates the direct depolarization of these neurons. A role for the mGluR1 found at postsynaptic sites in the STN remains to be determined. In addition to directly depolarizing the STN neurons, group I mGluR activation also has been demonstrated to increase the frequency of STN burst firing [20,21,22]. Since the switch from single spike activity to a burst-firing mode is one of the characteristics of parkinsonian states in

animal models[23-25] and parkinsonian patients [26,27], this effect may play a key role in the neuropathology of this disease.

THE SUBTHALAMO-NIGRAL SYNAPSE

Glutamatergic projections from the STN to the BG output nuclei constitute the final synapse in the indirect pathway. To date, the only study of mGluRs in the EPN has been an *in situ* study, and the results closely parallel findings in the SNr [13]. Therefore, we will focus on studies of the STN-SNr synapse. Neurons in the STN express mRNA for mGluR1, 2, 3, and 5, and the SNr GABAergic neurons express mRNA for mGluR1, 3, and 5[13]. Immunocytochemical studies have demonstrated presynaptic localization of mGluR2/3 [28], and 7 [15,16] at asymmetric synapses in the SNr. The presence of mGluR2 is of particular interest because it exhibits a rather restricted distribution in the BG. In addition to the STN, the only other BG cells found to express mGluR2 are the striatal cholinergic interneurons [13]. Therefore, compounds selective for mGluR2 would be expected to exhibit relatively few side effects. The group I mGluRs have been found postsynaptically localized at symmetric and asymmetric synapses in the SNr [17,29].

Several recent studies have provided a great deal of information on the physiological roles mGluRs play in regulating the STN-SNr synapse. Both group II and group III receptors have been shown to inhibit glutamatergic transmission at this synapse [28,30]. In accord with the immunocytochemical studies, the pharmacology and physiology of this inhibition is consistent with actions on presynaptic mGluR2/3 and 7 [28]. Activation of group I mGluRs produces a robust direct depolarization of SNr GABAergic neurons [29]. This effect is blocked by the mGluR1-selective antagonist CPCCOEt, but not by the mGluR5-selective MPEP. Therefore, in contrast to the effect of group I mGluR agonists in the STN, this effect appears to be mediated solely by mGluR1. Interestingly, stimulation of glutamatergic afferents in the SNr at frequencies consistent with the normal firing rate of STN neurons induces an mGluR-mediated slow EPSP which is completely blocked by CPCCOEt [29]. This indicates that postsynaptic mGluR1 may play an important role in tonic regulation of basal ganglia output.

Since increased activity in the STN is believed to play a key role in the pathophysiology of PD [5], the STN-SNr synapse is a logical site to target pharmacological interventions. The findings that the group II mGluRs are effective at decreasing transmission at this synapse, and exhibit a somewhat restricted distribution, indicate that these receptors could provide an ideal target for the development of antiparkinsonian compounds. Consistent with this, recent studies have demonstrated that the systemic injection of the highly selective group II mGluR agonist LY354740 decreases haloperidol-induced muscle rigidity [31] and catalepsy [30] in a rat model of PD.

METABOTROPIC GLUTAMATE RECEPTORS IN OTHER BASAL GANGLIA REGIONS

While this review has focused on the indirect pathway, it should be noted that mGluRs are expressed throughout the BG and have functional relevance at multiple sites. For example, input to the BG at the cortico-striatal synapse is modulated both presynaptically by group II and III mGluRs [14-17] and postsynaptically by group I mGluRs [17,32]. The main effect of the presynaptic mGluRs is to reduce the cortical input to the striatum [33-35]. Activation of the postsynaptic group I mGluRs produce a direct excitation of the indirect pathway[36,37]. Interestingly, mGluR5 has been found to exclusively colocalize with enkephalin in striatal

medium aspiny neurons [32] indicating that the selective activation of the indirect pathway may be mediated by this receptor.

The group III mGluRs mGluR4, and 7 have been localized to presynaptic symmetric striato-nigral terminals [14-16]. Activation of group I and III mGluRs decreases inhibitory transmission in the SNr [38], demonstrating that the mGluRs also play a role in modulating the direct pathway. In the case of the group I mGluRs, this is of particular interest since, as discussed above, mGluR1 has been demonstrated to directly activate SNr neurons. This direct excitation coupled with a group I-mediated disinhibition suggests that group I receptor activation could dramatically increase SNr output. Therefore, in addition to the relevance for PD, compounds selective for the group I mGluRs may hold therapeutic relevance for disorders involving alteration of activity through the direct pathway such as Huntington's disease, Tourette's syndrome, and epilepsy.

Finally, all three groups of mGluRs have been shown to modulate glutamatergic transmission in the substantia nigra pars compacta [39]. This finding is of particular interest since glutamate release in the SNc is hypothesised to play a role in the degeneration of the nigro-striatal dopamine system. While the source of the excitatory afferents regulated by mGluRs in SNc was not defined in these studies, it is likely that these EPSCs are mediated in part by activity at STN terminals. These data raise the exciting possibility that group II mGluR agonists have potential not only for reducing the symptoms of established PD, but could also slow progression of PD. Future studies will be needed to clearly define the role of increased STN activity in contributing to progression of the disorder and to rigorously define the mGluR subtypes involved in regulating transmission at STN-SNc synapses.

In summary, the mGluRs are expressed throughout the indirect pathway and selectively modulate synaptic transmission and cell excitability at each synapse in the pathway (table 1). Studies of this family of receptors not only provides insight to BG function, but holds promise for the development of therapeutic compounds for the treatment of movement disorders.

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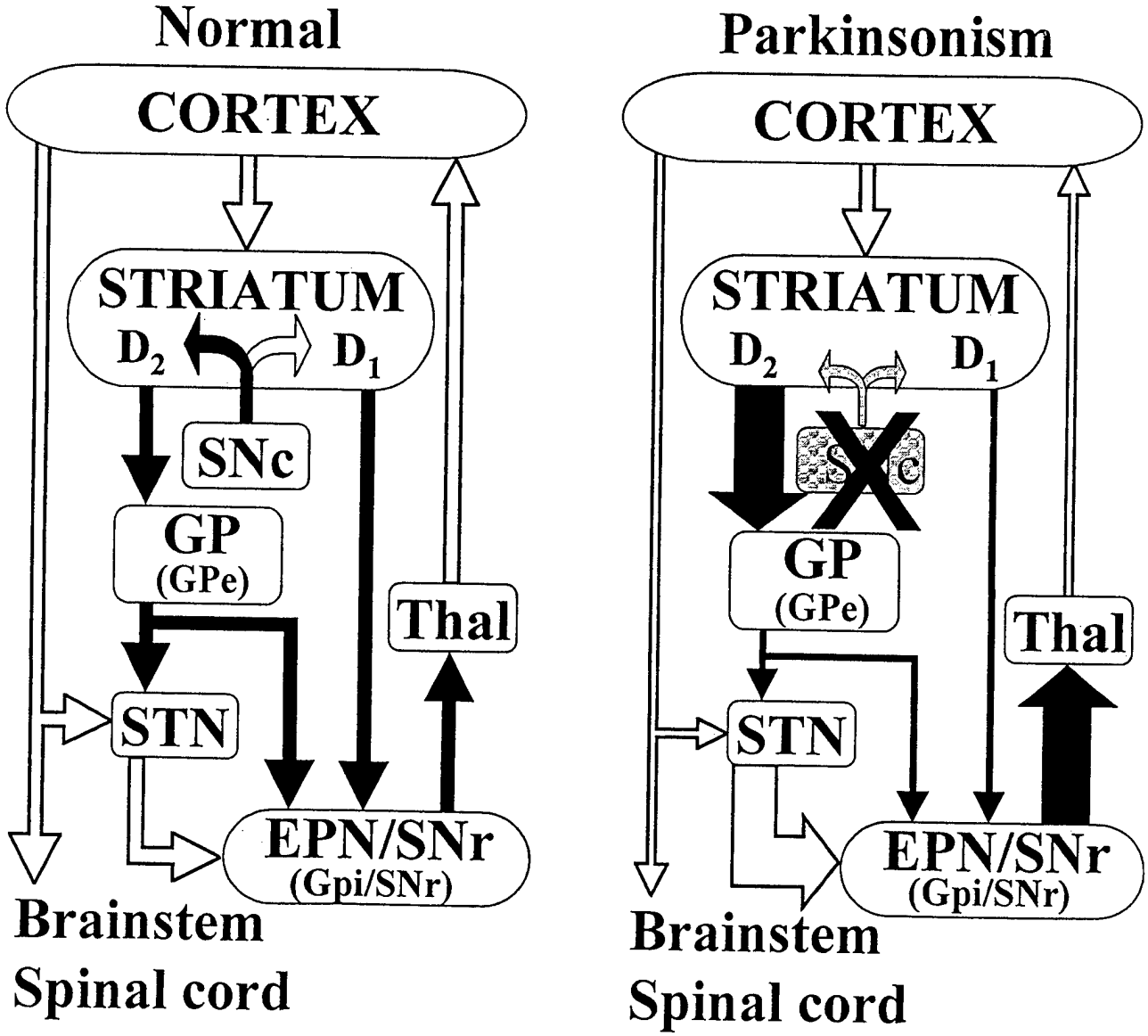

Figure 1.
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Table 1. Summary of distribution and physiological effects of mGluRs in the indirect pathway. Numbers are indicated for mGluR subtypes detected at the mRNA or protein level. The mRNA columns refer to mRNA expression in the neurons of origin for the presynaptic terminals, and the target neurons for the postsynaptic terminals. See text for references.

PRESYNAPTIC LOCALIZATION AND EFFECTS

Synapse	mRNA (Presynaptic cells)	Protein (Presynaptic Terminal)	Physiological Effect
Striato-pallidal	1, 3 , 4, 5	4, 7	?
Pallido-subthalamic	1, 5	?	?
Subthalamo-nigral	1, 2, 3, 5	2/3, 4, 7	Decrease Glutamate Release

POSTSYNAPTIC LOCALIZATION AND EFFECTS

Synapse	mRNA (Postsynaptic cells)	Protein (Postsynaptic Terminal)	Physiological Effect
Striato-pallidal	1, 5	1, 5, 7	?
Pallido-subthalamic	1, 2, 3, 5	1, 5	Direct Depolarization
Subthalamo-nigral	1, 3, 5	1, 5	Direct Depolarization

APPENDIX V

FENS
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3rd choice: excitatory amino acids and their receptors

PHYSIOLOGICAL ROLES OF MULTIPLE METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES IN RAT BASAL GANGLIA

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The primary pathophysiological change giving rise to the symptoms of Parkinson's disease (PD) is loss of substantia nigra dopaminergic neurons that are involved in modulating function of the striatum and other basal ganglia structures. Unfortunately, traditional therapies for treatment of PD based on dopamine replacement strategies eventually fail in most patients. Because of this, a great deal of effort has been focused on developing a detailed understanding of the circuitry and function of the basal ganglia in hopes of developing novel therapeutic approaches for restoring normal basal ganglia function in patients suffering from PD. We have performed a series of studies of the distribution and function of mGluR subtypes in the basal ganglia that suggest that members of this receptor family could serve as targets for novel therapeutic agents that would be effective in treatment of PD. For instance, we found that two group III mGluRs (mGluR4 and mGluR7) are localized on presynaptic terminals of striatal neurons in the globus pallidus where they could reduce GABA release. Furthermore, activation of group I mGluRs results in a depolarization and increased cell firing of neurons in the subthalamic nucleus (STN) and projection neurons of the substantia nigra pars reticulata (SNpr). Interestingly, studies with subtype-selective agonists and antagonists suggest that this effect is mediated by

mGluR1 in SNpr projection neurons and mGluR5 in STN neurons. Finally, we found that activation of group II mGluRs results in inhibition of glutamate release from STN terminals in the SNpr. Furthermore, selective agonists of group II mGluRs inhibit haloperidol-induced catalepsy in rats, suggesting an antiparkinsonian effect of these compounds. The rich distribution and diverse physiological roles of mGluRs in basal ganglia raises the possibility that these receptors may provide targets for novel therapeutic agents that could be used for treatment of PD and related disorders.

Oral presentation preferred

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METABOTROPIC GLUTAMATE RECEPTORS MODULATE EXCITATORY TRANSMISSION IN THE RAT SUBTHALAMIC NUCLEUS. H. Awad*, P.J. Conn. *Department of Pharmacology, Emory University School of Medicine, Graduate Program in Molecular and System Pharmacology, Atlanta, GA*

Overactivity in neurons of the subthalamic nucleus (STN) has been shown to be a major pathophysiological change that occurs in Parkinson's disease. Agents that reduce the activity of the STN may have beneficial therapeutic effects in Parkinson's disease. The primary afferents to the STN arise from the cortex, globus pallidus, thalamus and pedunculopontine nucleus (PPN). Here we examine the role of metabotropic glutamate receptors (mGluRs) in modulation of excitatory and inhibitory synaptic transmission in the STN. Electrophysiological recordings were made from STN neurons in parasaggital rat brain slices. Stimulating electrodes were placed in the internal capsule (IC) for stimulating descending afferents and in the cerebral peduncle (CP) for stimulating ascending afferents. Excitatory post-synaptic currents (EPSCs) were elicited in the STN in the presence of 10μM Bicuculline, and inhibitory post-synaptic currents (IPSCs) were elicited in the presence of 20μM CNQX and 20μM D-AP5. Under IC stimulation, the group I selective mGluR agonist DHPG (100μM) caused a 34.3±3.3% reduction of EPSCs, the group II agonist LY354740 (100nM) caused a 43.5±6.8% reduction in EPSCs, and the group III agonist L-AP4 (1mM) caused a 80.9±6.7% reduction in EPSCs. On the other hand, only group I and III mGluR activation, but not group II, caused a reduction in EPSCs elicited by CP stimulation of ascending fibers into the STN. mGluR activation had no effect on IPSC amplitude in the STN. These data suggest differential effects of mGluR activation on synaptic transmission arising from different afferents onto STN neurons. We are currently further investigating this mGluR inhibition of EPSCs to determine whether this effect is due to a presynaptic or postsynaptic modulation. *Supported by: grants from NIH NINDS, and the U.S. Army.*

740.15

METABOTROPIC GLUTAMATE RECEPTORS MODULATE EXCITATORY AND INHIBITORY TRANSMISSION IN SUBSTANTIA NIGRA PARS RETICULATA.

M. Wittmann, M.J. Marino, S.T. Rouse*, P.J. Conn. *Dept. of Pharmacology, Emory University, Atlanta, GA*

The Substantia nigra pars reticulata (SNr) is one of the main output structures of the basal ganglia (BG), a highly connected set of subcortical nuclei. Cortical information is processed through the BG to the SNr via two major pathways. The direct pathway which provides a GABAergic inhibition of the SNr projection neurons and the indirect pathway projecting through globus pallidus (GP) and subthalamic nucleus (STN) and resulting in a glutamatergic excitation of the output neurons. A delicate balance between these two inputs is believed to be crucial for initiation of movement, and imbalance in this system underlies the pathophysiology of movement disorders. Behavioral and physiological studies have shown that metabotropic glutamate receptors (mGluRs) play important roles in regulation of BG function. We have previously shown that presynaptically localized group-II mGluRs inhibit excitatory transmission at the STN-SNr synapse. We have now investigated the role of other mGluR subtypes in the modulation of inhibitory and excitatory transmission in SNr. We have found that group-III mGluRs also inhibit EPSCs at the STN-SNr synapse and that group-I and group-III mGluRs decrease IPSCs in the SNr. Miniature IPSC studies and paired pulse studies indicate that these inhibitory effects on EPSCs and IPSCs in SNr neurons are mediated by presynaptic mechanisms. We are currently further investigating the modulation of presynaptic mGluR effects at the STN-SNr synapse. It is known that cyclic AMP analogs and forskolin, an activator of adenylyl cyclase, can inhibit the function of presynaptic group-II mGluRs. Preliminary data reveal that forskolin (50μM) inhibits the presynaptic effect of the group-II specific agonist LY354740 (100nM) at the STN-SNr synapse. Current work is aimed at further investigating this effect and its implications on BG function. *Supported by: NIH NINDS and the U.S. Army.*

740.16

FUNCTIONAL ROLES OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN THE SUBSTANTIA NIGRA PARS RETICULATA.

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Behavioral and physiological studies have shown that metabotropic glutamate receptors (mGluRs) play important roles in regulation of basal ganglia (BG) function. Furthermore, specific mGluR subtypes are differentially localized throughout the BG. The predominant postsynaptic mGluRs are the group I mGluRs (mGluR1 and mGluR5), while group II and group III mGluRs are often localized presynaptically. This would indicate that group I receptors are localized in a manner consistent with direct modulation of the excitability of projection neurons. Since the over excitation of substantia nigra pars reticulata (SNr) GABAergic neurons is believed to play a major role in the pathophysiology of Parkinson's disease, group I mGluRs may provide an important target for new therapeutic agents that could be useful for treatment of this disorder. However, little is known about the physiological roles mGluRs play in regulating the function of BG structures. Previously, we have shown that the group I mGluR agonist DHPG produces a direct depolarization of GABAergic projection neurons in the SNr. Pharmacological studies reveal that this DHPG-induced depolarization is mediated by mGluR1. This is somewhat surprising, as we have found that both mGluR1 and mGluR5 are localized postsynaptically in SNr projection neurons, and these receptors are known to couple to the same signal transduction systems. We now report that activation of group I mGluRs induces a potentiation of NMDA-receptor currents in SNr projection neurons. Preliminary results indicate that this effect is also mediated by mGluR1. Current studies are aimed at determining the physiological significance of this modulation, and at investigating the role mGluR5 receptor activation plays in these cells. *Supported by: NIH NS98011, RR00165, and grants from The U.S. Army, and The National Parkinson's Foundation.*

740.17

FUNCTIONAL ROLES OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS IN TWO NEURONAL POPULATIONS IN RAT GLOBUS PALLIDUS.

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Three types of neurons have been characterized in rat globus pallidus (GP) based on morphological and electrophysiological properties. Although all three types are GABAergic, they are thought to be functionally distinct. In this study, whole-cell patch clamp recording in brain slices was used to investigate the functional roles of group I metabotropic glutamate receptors (mGluRs) in the two predominant cell types in GP, type I and type II. Type II neurons were characterized by time and voltage-dependent inward rectification. These cells exhibited input resistance of 813±159 MΩ. Rebound depolarization was observed in 40% of these cells. Type I GP neurons were characterized by lack of time and voltage-dependent inward rectification. These cells exhibited significantly lower input resistance of 405±20 MΩ. (RS)-3,5- DHPG (1-100 μM) was used to show the functional presence of group I mGluRs in these neuronal subtypes. CPCCOEt (100 μM) and MPEP (10μM) were utilized to differentiate between mGluR1 and mGluR5, members of group I mGluRs. Type II GP neurons responded to 100 μM DHPG with a robust depolarization of 16.6±8.7 mV from rest. Type I GP neurons, on the other hand, did not respond to 100 uM DHPG (5±3.8mV). Our results provide evidence that, in addition to their differences in membrane properties, these two GP neuronal populations may differ in their responses to activation of group I mGluRs. *Supported by: NIH NIDS and the US Army..*

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METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED REGULATION OF EXCITATORY TRANSMISSION IN THE RAT SUBSTANTIA NIGRA PARS RETICULATA

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Behavioral and physiological studies have shown that metabotropic glutamate receptors (mGluRs) play important roles in regulation of basal ganglia (BG) function. Furthermore, specific mGluR subtypes are differentially localized throughout the BG. The predominant postsynaptic mGluRs are a group I mGluRs, while group II and III mGluRs are often localized presynaptically. However, little is known about the physiological roles mGluRs play in regulating the function of BG structures. The group II mGluRs (mGluR2 and mGluR3) are expressed in neurons in the subthalamic nucleus (STN) and these receptors have been shown to regulate glutamate release in other brain regions. This led us to postulate that group II mGluRs are presynaptically localized on STN terminals in the substantia nigra pars reticulata (SNr) and that activation of these receptors would reduce excitatory synaptic responses. We have found that activation of presynaptically localized group II mGluRs inhibits excitatory transmission at the STN-SNr synapse. This suggests that a selective group II mGluR agonist could ameliorate the motor dysfunction associated with Parkinson's disease. Consistent with this, we find that the highly selective group II mGluR agonist LY354740 reverses catalepsy in an animal model of Parkinson's disease. In addition, the group I mGluRs (mGluR1 and mGluR5) are localized postsynaptically in SNr projection neurons. Here we demonstrate that activation of postsynaptically localized mGluR1 by exogenous agonists or synaptic glutamate produces a direct depolarization of GABAergic projection neurons in the SNr along with a concomitant increase in input resistance. In addition, activation of mGluR1 induces a potentiation of NMDA-receptor currents in SNr projection neurons. The fact that mGluR1 alone mediates these responses is somewhat surprising, as both mGluR1 and mGluR5 are known to couple to the same signal transduction systems. Since the over excitation of SNr GABAergic neurons is believed to play a major role in the pathophysiology of Parkinson's disease, mGluR1 may provide an important target for new therapeutic agents that could be useful for treatment of this disorder *Supported by grants from NIH, US Army, and the National Parkinson's Foundation.* *Animals were anesthetized with chloral hydrate (700 mg/kg, IP) prior to decapitation.*

American College of Neuropsychopharmacology Annual Mtg 12/10-14

DOPAMINE REGULATION OF METABOTROPIC GLUTAMATE RECEPTOR SIGNALING IN THE RAT SUBSTANTIA NIGRA PARS RETICULATA.

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The substantia nigra pars reticulata (SNr) is a primary output nucleus of the basal ganglia motor circuit Alterations in inhibitory and excitatory transmission in the basal ganglia output nuclei are known to play a major role in a variety of movement disorders. We have found that metabotropic glutamate receptors (mGluRs) play important roles in modulating transmission and neuronal excitability in the SNr. For example, mGluR1 is postsynaptically localized in GABAergic SNr neurons and activation of this receptor with exogenous agonists or synaptic activation induces a direct depolarization of SNr neurons. In addition, activation of presynaptic group I and group III mGluRs enhances SNr activity by decreasing inhibitory synaptic transmission. This combination of postsynaptic excitation, and disinhibition could exert a powerful excitatory influence on SNr output. Therefore, regulation of the mGluRs by alterations in the dopamine system could have broad implications for regulating basal ganglia function in both normal and pathophysiological conditions. Interestingly, we found that in vivo treatment of rats with the dopamine receptor antagonist haloperidol produces an enhanced response of SNr neurons to group I mGluR activation that can be measured in midbrain slices 1 day after initiation of haloperidol treatment. This effect is mediated by expression of an mGluR5-mediated response that is not present in control rats. In addition, activation of cyclic AMP-dependent protein kinase inhibits the group III mGluR-mediated disinhibition of SNr neurons. It is possible that D1 dopamine receptors that are present on these nerve terminals could enhance synaptic inhibition in the SNr by a cAMP-mediated reduction in presynaptic mGluR function.
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Activation of Group II Metabotropic Glutamate Receptors Inhibits Synaptic Excitation of the Substantia Nigra Pars Reticulata

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Loss of nigrostriatal dopaminergic neurons in Parkinson's disease (PD) leads to increased activity of glutamatergic neurons in the subthalamic nucleus (STN). Recent studies reveal that the resultant increase in STN-induced excitation of basal ganglia output nuclei is responsible for the disabling motor impairment characteristic of PD. On the basis of this, it is possible that any manipulation that reduces activity at excitatory STN synapses onto basal ganglia output nuclei could be useful in the treatment of PD. We now report that group II metabotropic glutamate receptors (mGluRs) are presynaptically localized on STN terminals and that activation of these receptors inhibits excitatory transmission at STN synapses. In agreement with the hypothesis that this could provide a therapeutic benefit in PD, a selective agonist of group II mGluRs induces a dramatic reversal of catalepsy in a rat model of PD. These results raise the exciting possibility that selective agonists of group II mGluRs could provide an entirely new approach to the treatment of PD. These novel therapeutic agents would provide a noninvasive pharmacological treatment that does not involve the manipulation of dopaminergic systems, thus avoiding the problems associated with current therapies.

Key words: substantia nigra pars reticulata; subthalamic nucleus; group II metabotropic glutamate receptors; Parkinson's disease; catalepsy; presynaptic inhibition

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by disabling motor impairments including tremor, rigidity, and bradykinesia. The primary pathological change giving rise to the symptoms of PD is the loss of dopaminergic neurons in the substantia nigra pars compacta that modulate the function of neurons in the striatum and other nuclei in the basal ganglia (BG) motor circuit. Currently, the most effective pharmacological agents for the treatment of PD include levodopa (L-DOPA), the immediate precursor of dopamine, and other drugs that replace the lost dopaminergic modulation of BG function (Poewe and Granata, 1997). Unfortunately, dopamine replacement therapy ultimately fails in most patients because of loss of efficacy with progression of the disease and severe motor and psychiatric side effects (Poewe et al., 1986). Because of this, a great deal of effort has been focused on developing new approaches for the treatment of PD.

Recent studies reveal that loss of nigrostriatal dopamine neurons results in a series of neurophysiological changes that lead to overactivity of a critical nucleus in the BG motor circuit termed the subthalamic nucleus (STN). The STN contains glutamatergic projection neurons that provide the major excitatory input to the globus pallidus internal segment (GPi) and the substantia nigra pars reticulata (SNr), the major output nuclei of the basal ganglia. Increased activity of STN neurons leads to an increase in glutamate release at STN synapses onto GABAergic projection neurons in the output nuclei. This glutamate-mediated overexcitation of BG output ultimately leads to a "shutdown" of thalamocortical projections and produces the motor impairment characteristic of PD (Wichmann and DeLong, 1997). Discovery of the pivotal role of increased STN-mediated excitation of the BG output nuclei in PD has led to a major focus on surgical approaches for treatment. For instance, lesions or high-frequency stimulation of the STN provides a therapeutic benefit to PD patients (Limousin et al., 1995). In addition, pallidotomy, a surgical lesion of the GP, produces similar therapeutic effects by reversing the impact of increased activity of STN neurons (Laitinen et al., 1992; Baron et al., 1996). Development of these highly effective neurosurgical approaches provides a major advance in our understanding of the pathophysiology of Parkinson's disease. However, surgical approaches are not widely available to Parkinson's patients. Because of their invasive nature, high cost, and the considerable expertise required, such treatment is reserved for patients that are refractory to dopamimetic therapy.

An alternative to surgical approaches to reducing the increased excitation of basal ganglia output nuclei in PD patients would be to use pharmacological agents that counteract the effects of overactivation of the STN neurons by reducing transmission at excitatory STN synapses onto the SNr and GPi neurons. Although antagonists of postsynaptic ionotropic glutamate receptors can improve parkinsonian symptoms in PD patients and in animal models of PD (Klockgether et al., 1993; Kornhuber et al., 1994), these compounds are most effective as adjuncts to dopamine replacement therapy (Starr, 1995). Another approach would be to target metabotropic glutamate receptors (mGluRs), which are often localized presynaptically on glutamatergic terminals where

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S.R.B. and M.J.M. contributed equally to this work.

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they can inhibit glutamate release. Interestingly, the group II mGluRs (mGluR2 and mGluR3) are expressed in STN neurons (Testa et al., 1994), and these receptors have been shown to regulate glutamate release in other brain regions (Hayashi et al., 1993; Shigemoto et al., 1997). We now report that group II mGluRs are presynaptically localized on STN terminals in the SNr and that activation of these receptors reduces excitatory synaptic responses. Furthermore, activation of group II mGluRs provides a dramatic therapeutic effect in a rat model of Parkinson's disease. If this or related drugs prove to be effective in patients with Parkinson's disease, this could lead to a novel approach for the treatment of this debilitating disorder.

MATERIALS AND METHODS

Materials. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), (*R,S*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG), D(−)-2-amino-5-phosphonopentanoic acid (D-AP-5), and (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) were obtained from Tocris (Ballwin, MO). 2*R*,4*R*-4-Aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC), (+)-2-aminobicyclo[3·1·0]-hexane-2,6-dicarboxylate monohydrate (LY354740), and 2*S*-2-amino-2-(1*S*,2*S*-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495) were gifts from D. Schoepp and J. Monn (Eli Lilly, Indianapolis, IN). All other materials were obtained from Sigma (St. Louis, MO).

Electrophysiology. Whole-cell patch-clamp recordings were obtained as described previously (Marino et al., 1998) except that recordings were made under visual control. Fifteen- to 18-d-old Sprague Dawley rats were used for all patch-clamp studies. Brains were rapidly removed and submerged in an ice-cold sucrose buffer (in mM): sucrose, 187; KCl, 3; $CaCl_2$, 2; $MgSO_4$, 1.9; KH_2PO_4, 1.2; glucose, 20; and $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2. Parasagittal slices (300 μm thick) were made using a Vibraslicer (WPI). Slices were transferred to a holding chamber containing normal artificial CSF (ACSF; in mM, NaCl, 124; KCl, 2.5; $MgSO_4$, 1.3; NaH_2PO_4,1.0; $CaCl_2$, 2.0; glucose, 20; and $NaHCO_3$, 26; equilibrated with 95% O_2/5% CO_2). In some experiments, 5 μM glutathione, 500 μM pyruvate, and 250 μM kynurenate were included in the sucrose buffer and holding chamber. These additional compounds tended to increase slice viability but did not have any effect on experimental outcome. Therefore all of the data from these two groups have been pooled. Slices were transferred to the stage of a Hoffman modulation contrast microscope and continually perfused with room temperature ACSF (~3 ml/min; 23–24°C). Neurons in the substantia nigra pars reticulata were visualized with a 40× water immersion lens. Patch electrodes were pulled from borosilicate glass on a Narashige vertical patch pipette puller (Tokyo, Japan) and filled with buffer (in mM, potassium gluconate, 140; HEPES, 10: NaCl, 10; EGTA, 0.6; GTP, 0.2; and ATP, 2; pH adjusted to 7.5 with 0.5N NaOH). Biocytin (0.5%; free base) was added just before use. Electrode resistance was 3–7 M[SCAP]Ω. For measurement of synaptically evoked currents, bipolar tungsten electrodes were used to apply stimuli to the STN. Stimulating electrodes were positioned with one pole slightly penetrating the tissue and the other pole above the slice. Synaptically evoked EPSCs were record from a holding potential of −60 mV, and slices were bathed in 50 μM picrotoxin. IPSCs were evoked in a similar manner but with the electrodes placed in the cerebral peduncle rostral to the recording sight and in the presence of 10 μM CNQX and 10 μM D-AP-5 to block excitatory transmission. IPSCs were recorded from a holding potential of −50 mV. STN-evoked fiber volleys were recorded by placing a low-resistance pipette (0.5–2 M[SCAP]Ω) filled with 3 M NaCl in the cerebral peduncle and stimulating the STN as described above. Fiber volleys were evoked in the presence of 20 μM CNQX and 20 μM bicuculline. For measurement of kainate-evoked currents, kainate (100 μM) was pressure ejected into the slice from a low-resistance pipette. Kainate-evoked currents were recorded from a holding potential of −60 mV, and slices were bathed in ACSF containing 500 nM tetrodotoxin. For studies of miniature EPSCs (mEPSCs), slices were bathed in standard ACSF with the addition of 50 mM mannitol, 500 nM tetrodotoxin, and 10 μM bicuculline warmed to 25°C. Glutamate-evoked EPSCs were recorded in the presence of 20 μM bicuculline. Glutamate (100 μM in ACSF) was applied by a syringe pump (1 ml/min) through a microapplicator made from a fused silica microtube (MicroFil; WPI). The microapplicator was positioned slightly above the slice and dorsal to the STN. The flow of glutamate was parallel to the bath flow, and the slice was arranged so that glutamate application to surrounding areas was minimized (see Fig. 3). This method was also used to produce a local application of LY345740 for some experiments (see Fig. 1*A,B*). GABAergic projection neurons were identified according to previously established electrophysiological and morphological criteria (Richards et al., 1997). GABAergic neurons exhibited spontaneous repetitive firings, short-duration action potentials (half-amplitude duration = 1.7 ± 0.2 msec), little spike frequency adaptation, and a lack of inward rectification, whereas dopaminergic neurons displayed no, or low-frequency, spontaneous firings, longer-duration action potentials (half-amplitude duration = 7.0 ± 0.5 msec), strong spike frequency adaptation, and a pronounced inward rectification. Light microscopic examination of biocytin-filled neurons indicated that GABAergic neurons had extensive dendritic arborizations close to the cell body, whereas the dendritic structures of dopaminergic neurons were relatively sparse. All of the data presented in these studies are from confirmed GABAergic neurons.

Immunocytochemistry. Preparation of the tissue for immunocytochemical analysis at the electron microscopy level followed previously published protocols (Bradley et al., 1996). The avidin–biotin–peroxidase method (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA) was used to detect mGluR2/3 immunoreactivity in rat (n = 2) SNr. The peroxidase reaction was developed in 0.05% diaminobenzidine and 0.01% H_2O_2. Antibodies that specifically recognize mGluR2 and mGluR3 are from Chemicon (Temecula, CA).

Behavioral studies. Male Sprague Dawley rats 30 d old at the start of experiments were injected intraperitoneally with either haloperidol (2 mg/ml solution dissolved in 8.5% lactic acid, neutralized with 1N NaOH, and diluted to 0.3 mg/ml in saline) or saline and returned to their home cage for 30 min. After 30 min, the animals were injected with either saline or LY354740 (0.6–6 mg/ml dissolved in saline). Catalepsy was measured 1 hr later by placing the animal's forepaws on a bar elevated 4.5 cm. The time to removal of one paw was measured by a stopwatch. Animals were then placed on a vertical mesh ~6 inches above the ground, and the time to remove one paw from the mesh was measured. Animals were tested once per day, and saline controls were run between each drug test. All animals were habituated to the tasks by 3 consecutive days of saline control treatment before beginning drug testing.

RESULTS

Whole-cell patch-clamp techniques were used to record EPSCs from GABAergic projection neurons of the SNr in midbrain slices. EPSCs were elicited by stimulation of the STN with bipolar stimulating electrodes (0.4–12.0 μA every 60–90 sec) in the presence of 50 μM picrotoxin. EPSCs elicited with this protocol had a constant latency, were monophasic, and were completely abolished with the application of 10 μM CNQX (n = 10; data not shown), suggesting that the synaptic response was a monosynaptic glutamatergic EPSC.

Activation of group II mGluRs inhibits transmission at the STN–SNr synapse

Brief local application of 100 nM LY354740, a highly selective agonist of group II mGluRs (Monn et al., 1997; Schoepp et al., 1997), produced a reversible depression of EPSCs in SNr projection neurons (Fig. 1*A,B*). It should be noted, whereas LY354740 reduced evoked EPSCs in every cell tested, that longer bath applications resulted in inconsistent washout of the effect of LY354740. This is primarily because we recorded from cells at different depths in the slice. The deeper cells required longer periods of agonist application and exhibited slower washout of effects. However, longer bath applications were used in all additional studies to ensure an equilibrium concentration of drug at the sites of action and a maximal response. A concentration–response curve for LY354740 revealed an EC_{50} of ~75 nM (Fig. 1*C*), consistent with the potency of this compound at group II mGluRs. The steep slope of the concentration–response curve for LY354740 is consistent with the dose–response relationship reported for a number other effects of this drug in both recom-

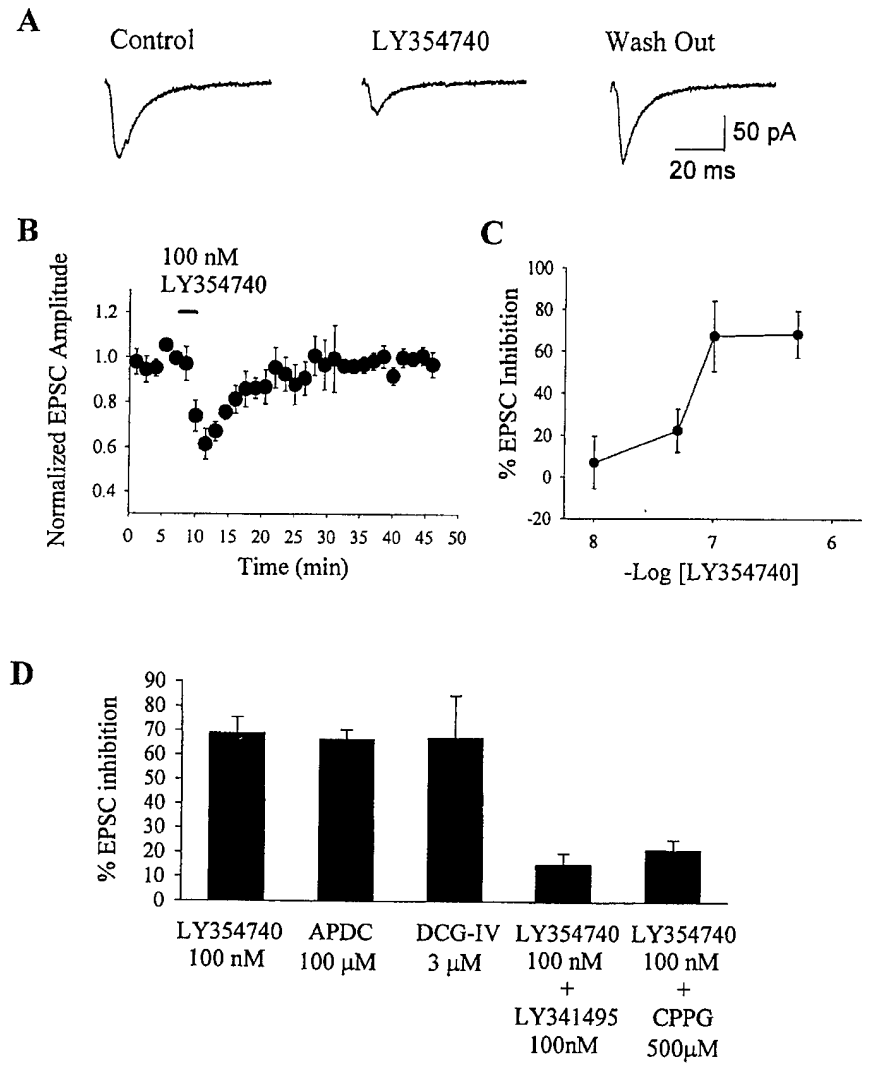

Figure 1. Activation of group II mGluRs reduces EPSCs at the STN–SNr synapse. *A*, Evoked EPSCs before (*Control*), during (*LY354740*), and after (*Wash Out*) a brief local application of LY354740. Applications of LY354740 dramatically reduce EPSCs, and this effect is reversible. *B*, Average time course of the effect of 100 nM LY354740 (application indicated by *horizontal bar*). Each *point* represents the mean (± SEM) of data from five cells. *C*, Dose–response relationship of LY354740-induced inhibition of EPSCs. The effect of inhibition of EPSCs is maximal at 100 nM. Each *point* represents the mean of three experiments. *D*, Effects of specific group II mGluR agonists on EPSCs at the STN–SNr synapse and block of the LY354740-induced inhibition of EPSCs by application of group II mGluR antagonists before application of the agonist. Agonists include LY354740 (100 nM), APDC (100 μM), and DCG-IV (3 μM). Antagonists include LY341495 (100 nM) and CPPG (500 μM). Each *vertical bar* represents the mean (± SEM) of data collected from five cells ($^*p < 0.01$).

binant and native systems (Monn et al., 1997; Schaffhauser et al., 1997; Schoepp et al., 1997). The reduction of EPSC amplitude was mimicked by two other highly selective agonists of group II mGluRs, 2*R*,4*R*-APDC (Schoepp et al., 1995) and DCG-IV (Hayashi et al., 1993; Gereau and Conn, 1995a) (Fig. 1*D*), and was completely blocked by previous application of LY341495 (100 nM) or CPPG (500 μM) (Fig. 1*D*), both of which are antagonists active at group II mGluRs (Toms et al., 1996; Kingston et al., 1998).

Group II mGluRs are localized presynaptically at excitatory terminals in the SNr

Taken together, these data suggest that activation of group II mGluRs reduces transmission at the STN–SNr synapse. We used a combination of immunocytochemical and biophysical approaches to determine whether group II mGluRs elicit this effect by a presynaptic or a postsynaptic mechanism of action. First, we used antibodies that specifically recognize both mGluR2 and mGluR3 for immunocytochemical localization of group II mGluRs in the SNr. Analysis of mGluR2/3 immunoreactivity at the electron microscopic level revealed that group II mGluRs are presynaptically localized (Fig. 2). The morphology of the labeled synapses, including their asymmetric nature, was characteristic of STN terminals (Fig. 2*A–D*) (Bevan et al., 1994). Quantification of the labeling was assessed by counting asymmetric terminals on three randomly selected grids that resulted in an estimated 30% labeling of asymmetric terminals (25 labeled terminal of 82 total). However, it is important to note that quantification of any preembedding immunocytochemical labeling at the electron microscopic level is confounded by the nonhomogeneous penetration of the antibodies through the vibratome sections. The first 5–10 μm on both sides of the sections are usually labeled, whereas the middle remains devoid of immunostaining. This implies that the lack of immunoreactivity in some structures could be attributable either to a genuine lack of antigens or to the inaccessibility of the antibodies to this particular site. Therefore, only the positive immunolabeling can be conclusively interpreted. Because of this, the 30% labeling observed in these studies represents a lower limit to the extent of staining. We also observed labeling of terminals that did not make clear synaptic contact with postsynaptic elements and of fine processes that were reminiscent of previous reports of mGluR2/3 distribution in preterminal axons

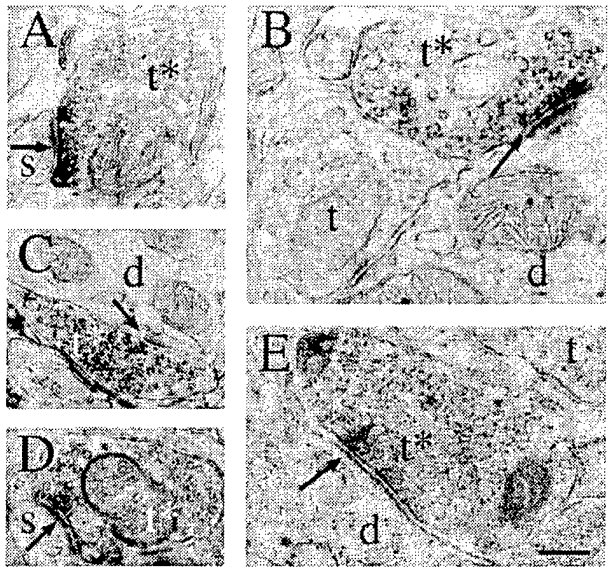

Figure 2. Group II mGluRs are presynaptically localized at asymmetric terminals in the SNr. *A–D,* Electron micrographs demonstrating presynaptic mGluR2/3 immunoreactivity at asymmetric terminals in the SNr. Labeled (*) axon terminals (*t*) are shown synapsing on unlabeled dendrites (*d*) and dendritic spines (*s*). *E,* An example of a labeled terminal forming a symmetric synapse. Synapses are indicated by *arrows*. Scale bar: *A*, 301 nm; *B*, 203 nm; *C*, 315 nm; *D*, 263 nm; *E*, 207 nm.

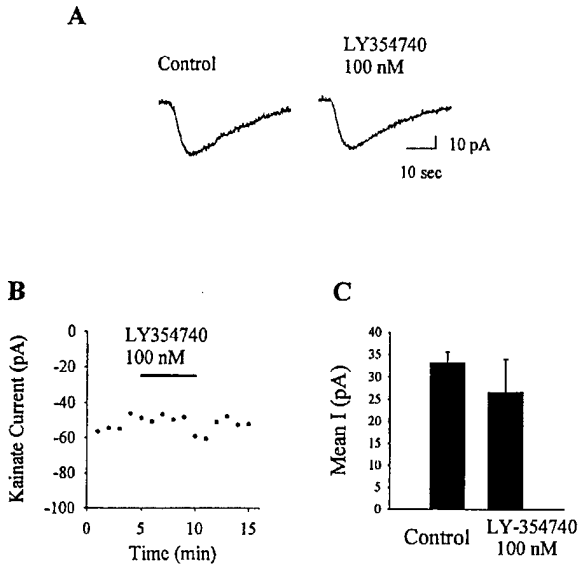

Figure 3. Activation of group II mGluRs has no effect on the response to exogenously applied kainate. *A,* Representative *traces* of kainate-evoked currents in the SNr projection neurons before (*Control; left*) and during application of 100 nM LY354740 (*right*). *B,* Time course of the effect of LY354740 on the amplitude of kainate-evoked currents. *C,* Mean data demonstrating the lack of effect of group II mGluR activation on kainate-evoked currents (mean ± SEM; $p > 0.05$; $n = 5$).

(data not shown) (Lujan et al., 1997). Furthermore, there was occasional labeling of symmetric synapses (Fig. 2*E*), although the majority of symmetric synapses were unlabeled. There was no observable staining of dendrites, dendritic spines, or other postsynaptic elements.

The group II mGluR-mediated inhibition of synaptic transmission is caused by a presynaptic mechanism

The presence of mGluR2/3 immunoreactivity at presynaptic but not postsynaptic sites in the SNr suggests that these receptors are likely to act by inhibiting glutamate release from presynaptic terminals rather than by modulating the postsynaptic glutamate-gated ion channels. To test this hypothesis further, we determined the effects of maximal concentrations of LY354740 on currents elicited by brief (50–500 msec) pressure ejection of kainate (100 μM) into the slice. In the presence of 500 nM tetrodotoxin, kainate application produced a robust, stable, inward current in SNr GABAergic neurons (Fig. 3*A*). The kainate-evoked currents were blocked by application of 10 μM CNQX ($n = 4$; data not shown) indicating that they were mediated by activation of AMPA/kainate receptors. Application of 100 nM LY354740 produced no significant change in kainate-evoked currents (Fig. 3*A,B*).

Although the lack of effect of LY354740 on kainate-evoked currents is consistent with a presynaptic mechanism of action, it is conceivable that exogenously applied kainate selectively activates nonsynaptic glutamate receptor channels and that LY354740 selectively modulates channels that are localized at synapses. Thus, we also determined the effect of maximal concentrations of LY354740 on the frequency and amplitude of spontaneous mEPSCs. Recordings were made in the presence of tetrodotoxin (500 nM) to block activity-dependent release and of bicuculline (10 μM) to block $GABA_A$-mediated synaptic currents. LY354740 (100 nM) produced no significant alteration in mEPSC frequency, amplitude, or waveform (Fig. 4*A–C*). This can be observed by a lack of effect of LY354740 on either the amplitude histograms (Fig. 4*C*) or the cumulative probability plots (Fig. 4*D*). Furthermore, overlay of an average of all mEPSCs before and after LY354740 application shows identical current amplitudes and kinetics between the two conditions (Fig. 4*B*). The average mEPSC frequency is 4.71 ± 0.79 Hz before drug application and 4.66 ± 0.8 Hz during application of 100 nM LY354740 ($p > 0.05$; $n = 5$). The average amplitude of mEPSCs was 9.2 ± 1.3 pA before and 8.4 ± 0.8 pA after LY354740 addition ($p > 0.05$; $n = 5$).

The lack of effect on mEPSC amplitude and frequency is consistent with the group II mGluR-mediated inhibition in synaptic transmission having a presynaptic site of action. There are a number of potential mechanisms by which a receptor could act presynaptically to reduce action potential-dependent release without decreasing the frequency of mEPSCs. For instance, mEPSCs are thought to be voltage independent and therefore should be insensitive to modulation of presynaptic voltage-dependent ion channels. If a receptor reduces transmission by inhibiting a presynaptic voltage-dependent calcium channel or increasing conductance through a voltage-dependent potassium channel rather than having some downstream effect on the release machinery, this may reduce evoked responses without affecting mEPSCs. This effect has been demonstrated at a variety of synapses where agents known to act presynaptically, such as cadmium, abolish evoked EPSCs but have no effect on either the frequency or amplitude of mEPSCs (Parfitt and Madison, 1993; Doze et al., 1995; Gereau and Conn, 1995b; Scanziani et al., 1995).

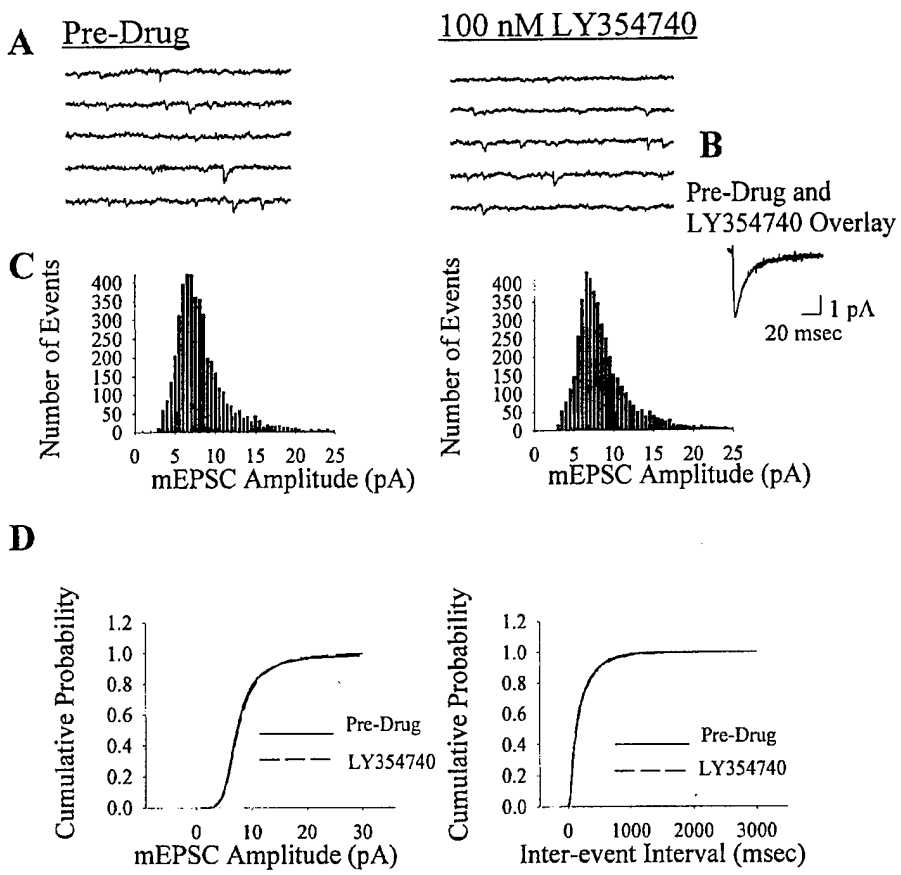

Figure 4. Inhibition of EPSCs at the STN–SNr synapse is mediated by a presynaptic mechanism. *A,* Examples of mEPSCs before (*Pre-Drug; left*) and during application of 100 nM LY354740 (*right*). *B,* Overlayed averages of all mEPSCs recorded before and during LY354740 application, demonstrating the lack of effect on the amplitude and kinetics of mEPSCs. *C,* Amplitude histograms of mEPSCs before (*left*) and during application of 100 nM LY354740 (*right*). *D,* Cumulative frequency plots illustrating the lack of effect of LY354740 on mEPSC amplitude (*left*) and inter-event interval (*right*) (Kolmogorov–Smirnov test; $p = 0.99$). The data shown are representative of five separate experiments.

Although the analysis of the effects of LY354740 on mEPSCs is consistent with a presynaptic site of action, one concern with studies of mEPSCs is that it is impossible to identify the source of afferent fibers. This issue is particularly important in cases in which there is no observable effect on mEPSC frequency because of the possibility that the majority of mEPSCs arise from a separate population of afferents than those stimulated to produce evoked release. Although the majority of glutamatergic input to the SNr arises from the STN, several other regions including the pedunculopontine nucleus (Charara et al., 1996) and the nucleus raphe (Corvaja et al., 1993) provide a sparse projection accounting for a small percentage of asymmetric terminals in the SNr that could release glutamate. To address this issue, we applied glutamate directly to the STN to produce a selective activation of STN cell bodies without exciting fibers of passage (Fig. 5*A*). Application of glutamate (100 μM) to the STN produced an increase in the frequency of spontaneous EPSCs recorded in SNr neurons (basal, 4.8 ± 1.6 Hz; glutamate, 12.4 ± 5.7 Hz; $n = 5$) without affecting spontaneous EPSC amplitude (basal, 9.6 ± 1.2 pA; glutamate, 9.4 ± 1.4 pA; $n = 5$). In agreement with a selective activation of cell bodies, movement of the glutamate application pipette slightly out of the STN to the cerebral peduncle had no effect on spontaneous EPSC frequency (ratio of glutamate/basal, application to STN, 3.0 ± 1.3; application to cerebral peduncle, 0.92 ± 0.1; $n = 3$) (Fig. 5*A*). To test for group II mGluR-mediated inhibition of transmission at the STN–SNr synapse, we determined the effects of maximal concentrations of LY354740 on the frequency and amplitude of glutamate-evoked EPSCs. In agreement with the electrical stimulation results, we found that activation of group II mGluRs significantly reduced the frequency of glutamate-evoked EPSCs without affecting the amplitude or kinetics of the response (Fig. 5*B–F*).

Taken together, these data strongly support the hypothesis that activation of group II mGluRs decreases transmission at the STN–SNr synapse by a presynaptic mechanism. However, it is possible that a group II mGluR agonist could reduce evoked EPSCs by a mechanism that does not directly involve regulation of synaptic transmission, such as inducing a decrease in the excitability of the STN neurons or decreasing axonal conductance. To examine the mechanism of this presynaptic modulation further, we assessed the effects of maximal concentrations of LY354740 on the excitability of STN neurons. Whole-cell current-clamp recordings from STN neurons during application of 100 nM LY354740 indicate that activation of group II mGluRs has no effect on membrane potential (control infusion $\Delta V_m = -0.93 \pm 0.34$ mV; $n = 4$; LY354740 infusion $\Delta V_m = -0.99 \pm 0.83$ mV; $n = 7$) (Fig. 6*A,D*) or input resistance (control, 671 ± 123.6 M[SCAP]Ω; LY354740, 665 ± 129.4 M[SCAP]Ω; $n = 3$) (Fig. 6*B,D*). We also applied a series of small depolarizing current injections to obtain an approximate estimate of the action potential threshold. Application of 100 nM LY345740 did not effect the lowest potential at which action potentials were observed (control infusion, −48.3 ± 1.28 mV; LY354740, −48.7 ± 1.55 mV; $n = 4$)

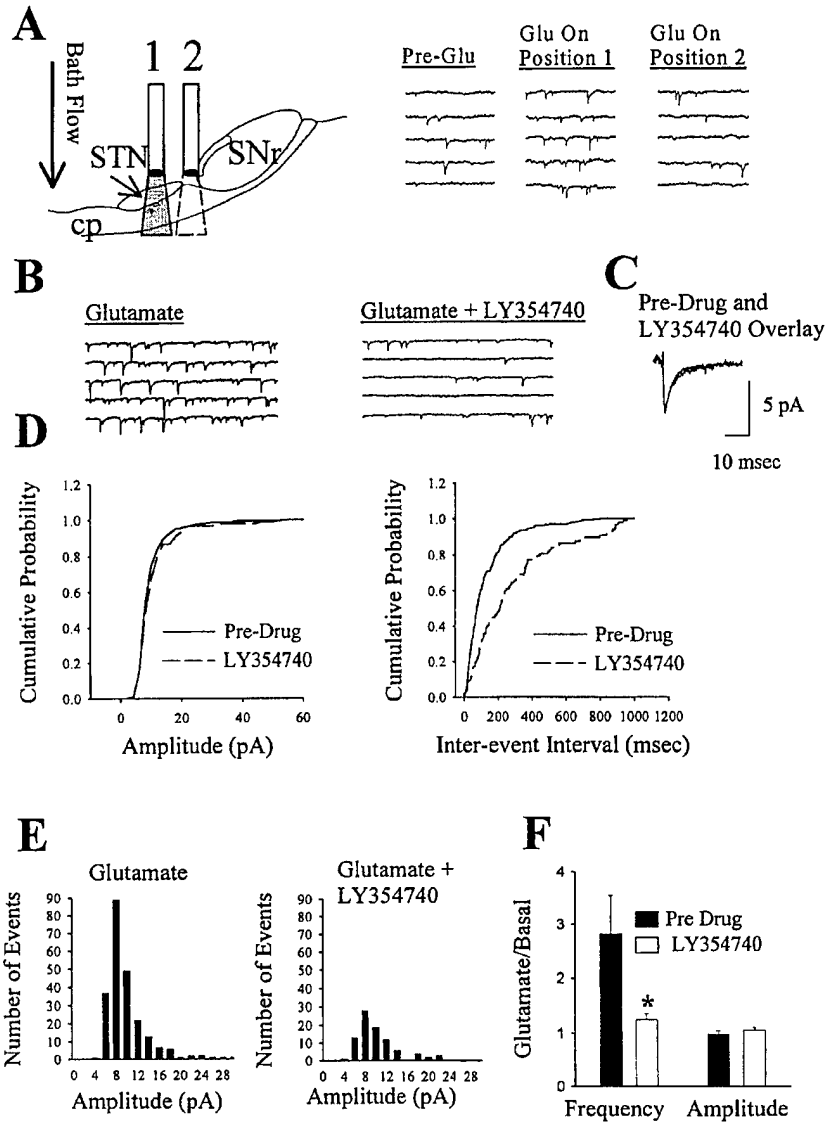

Figure 5. Activation of group II mGluRs reduces the frequency of EPSCs evoked by glutamate application to the STN. *A,* A demonstration of the experimental paradigm used. Direct application of glutamate (100 μM; 1 ml/min; 30 sec) to the STN produces approximately a threefold increase in EPSC frequency without affecting EPSC amplitude. Moving the microapplicator to a position *above* the cerebral peduncle (*cp*) produced no change in the frequency of EPSCs, indicating that the glutamate effect is caused by selective activation of STN neurons and not by fibers of passage. *B,* Examples of glutamate-evoked EPSCs both before (*left*) and during the application of 100 nM LY354740 (*right*). *C,* Overlayed *traces* of average glutamate-evoked EPSCs before and during 100 nM LY354740 application indicating no change in the amplitude or kinetics of the responses. *D,* Cumulative frequency plots illustrating a lack of effect of LY354740 on amplitude (*left*; Kolmogorov–Smirnov test; $p > 0.05$) and a significant increase in interevent interval (*right*; Kolmogorov–Smirnov test; $p < 0.01$), indicating that LY345740 selectively reduces the frequency of glutamate-evoked EPSCs. *E,* Frequency–amplitude histograms demonstrating a decrease in the frequency but no change in the mean amplitude of glutamate-evoked EPSCs. *F,* Mean (± SEM) data demonstrating that glutamate induces approximately a threefold increase in frequency over basal values without altering amplitude. This glutamate-evoked increase is significantly reduced by LY345740. Each *vertical bar* represents the mean (± SEM) of data collected from five cells (*$p < 0.05$).

(Fig. 6*C,D*). Therefore, these data indicate that the group II mGluR-mediated inhibition of transmission at the STN–SNr synapse cannot be explained by a decrease in the somatic excitability of the presynaptic neurons. We also recorded presynaptic fiber volleys by placing an extracellular recording electrode in the cerebral peduncle, the point of entry of STN fibers into the SNr, and electrically stimulating the STN. In the presence of blockers of fast glutamatergic (20 μM CNQX) and GABAergic (20 μM bicuculline) transmission, we recorded a robust negative inflection in the field that was sensitive to tetrodotoxin (500 nM), indicating that this is a measure of the firing of presynaptic axons (Fig. 6*E—G*). Application of 100 nM LY354740 had no effect on the presynaptic fiber volley, indicating that activation of group II mGluRs does not alter STN axonal excitability. Taken together, these data indicate that the group II mGluR-mediated reduction in transmission at the STN–SNr synapse is caused by a modulation of the presynaptic terminal or the preterminal axon.

Activation of group II mGluRs has no effect on inhibitory synaptic transmission in the SNr

If group II mGluRs selectively regulate transmission at STN synapses without altering transmission at inhibitory synapses in the SNr, agonists of these receptors would have a net inhibitory effect on excitatory drive through this portion of the basal ganglia circuit. The immunocytochemical data presented above suggest that mGluR2/3 immunoreactivity is not present on the majority of inhibitory synapses in the SNr, suggesting that group II mGluRs are not likely to modulate IPSCs in this region. To test this hypothesis directly, we determined the effect of LY354740 on evoked IPSCs recorded in SNr projection neurons. Consistent with previous reports (Radnikow and Misgeld, 1998), stimulation of the cerebral peduncle produced a robust, bicuculline-sensitive IPSC (Fig. 7). Application of a concentration of the group II mGluR agonist LY354740 that is maximally effective in reducing EPSCs had no effect on IPSC amplitude. These results suggest

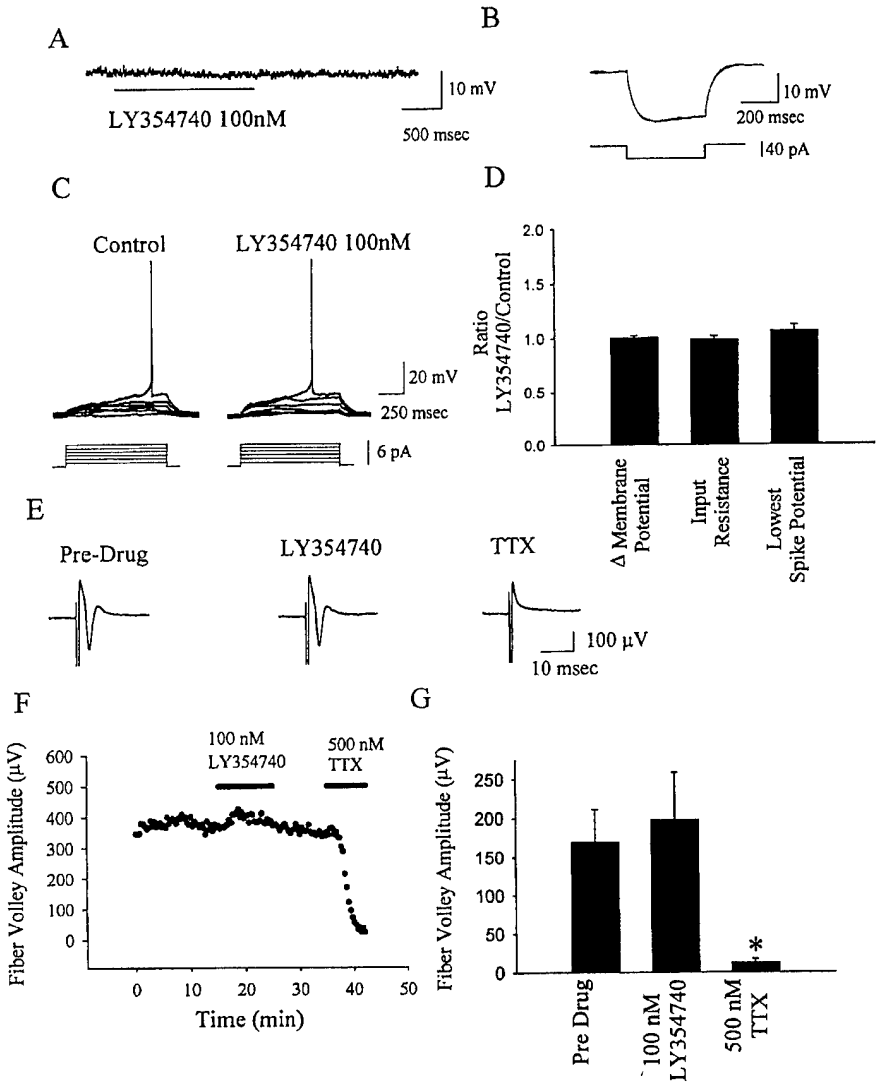

Figure 6. Activation of group II mGluRs does not effect the excitability of STN neurons. *A,* Representative current-clamp recording demonstrating that application of 100 nM LY345740 does not alter membrane potential. *B,* Overlayed *traces* of responses to the injection of hyperpolarizing current demonstrating that LY354740 has no effect on input resistance. *C,* Representative *traces* of experiments in which small depolarizing current injections were used to determine the lowest potential at which an STN neuron would produce an action potential. Application of 100 nM LY354740 has no effect on this potential. *D,* Mean (± SEM) of data demonstrating the lack of effect of group II mGluR activation on membrane potential, input resistance, or lowest spike potential. Data are from three to seven cells per condition. *E, F,* Representative *traces* (*E*) and time course (*F*) demonstrating that LY354740 does not alter presynaptic fiber volleys evoked by stimulation of the STN. *G,* Mean (± SEM) of data from four independent experiments demonstrating that activation of group II mGluRs has no effect on presynaptic fiber volleys. The fiber volley is blocked by the application of 500 nM TTX indicating that the volley is a measurement of presynaptic axonal action potential.

that agonists of group II mGluRs will selectively inhibit excitatory transmission through the indirect pathway to the SNr without impacting direct GABA-mediated inhibition of SNr neurons.

Activation of group II mGluRs exhibits antiparkinsonian effects

The preceding data clearly demonstrate that group II mGluRs mediate a presynaptic inhibition of transmission at the STN–SNr synapse. Because overactivity at this synapse is thought to contribute to the motor dysfunction associated with PD and other hypokinetic disorders, we tested the hypothesis that activation of group II mGluRs would increase mobility in a rat model of parkinsonism using haloperidol-induced catalepsy (Ossowska et al., 1990; Schmidt et al., 1997). Two standard behavioral measures were used to assess catalepsy in rats treated with the dopamine receptor antagonist haloperidol. First, the front paws of control and experimental rats were placed on a horizontal bar (4.5 cm high), and the latency to remove a paw from the bar was measured. Second, rats were placed on a vertical grid, and the latency to remove a paw from the grid was measured (Kronthaler and Schmidt, 1996). Consistent with previous reports (Ossowska et al., 1990; Schmidt et al., 1997), haloperidol (1.5 mg/kg) induced a robust catalepsy that could be observed as an increase in latency with both behavioral measures (Fig. 8). Interestingly, haloperidol-induced catalepsy was reversed in a dose-dependent manner by intraperitoneal injection of the group II mGluR agonist LY354740. Injection of LY354740 alone had no significant effect on these behavioral measures.

DISCUSSION

We have found that group II mGluRs are presynaptically localized on STN terminals in BG output nuclei where they reduce transmission at STN–SNr synapses. Furthermore, a selective agonist of group II mGluRs has behavioral effects in rats that are consistent with an antiparkinsonian action. These data suggest that activation of group II mGluRs restores the normal function of BG circuits by acting at a point downstream of the striatum where dopamine receptor blockade occurs.

The finding that LY354740 alone had no effect on measures of

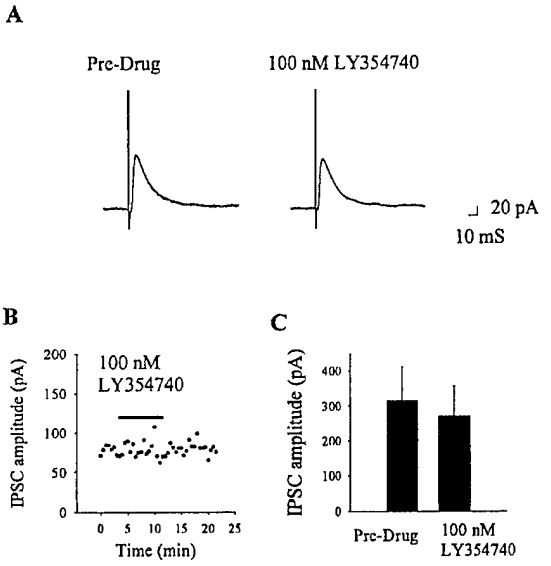

Figure 7. Activation of group II mGluRs has no effect on inhibitory transmission in the SNr. *A,* Representative *traces* of evoked IPSCs before (*Pre-Drug; left*) and during the application of 100 nM LY354740 (*right*). *B,* Time course of the effect of LY354740 on IPSC amplitude. *C,* Mean data demonstrating the lack of effect of group II mGluR activation on IPSC amplitude. Data represent the mean (± SEM) of seven separate experiments ($p > 0.05$).

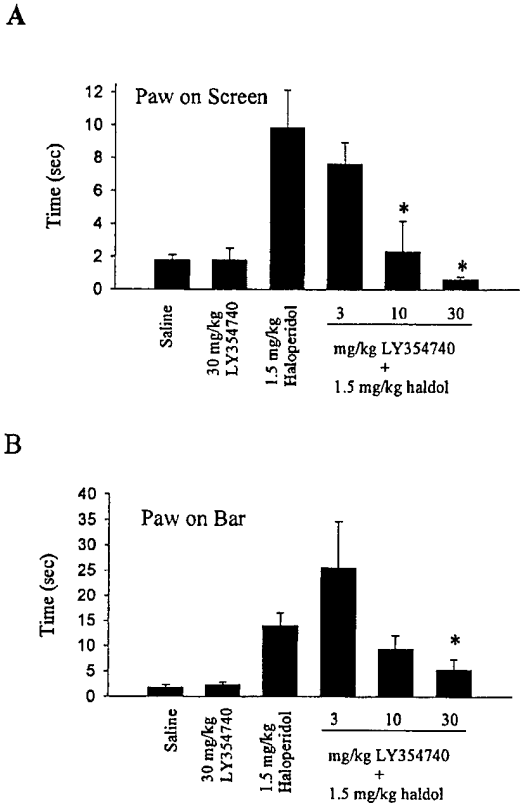

Figure 8. Activation of group II mGluRs reverses catalepsy in an animal model of Parkinson's disease. The degree of haloperidol-induced catalepsy was measured as either latency to the first paw movement when the animal was placed on a vertical grid (*A*) or latency to remove a paw from a bar (*B*). Haloperidol (1.5 mg/kg, i.p.) induces a pronounced catalepsy that was reversed in a dose-dependent manner by LY354740 (3–30 mg/kg, i.p.) (*$p < 0.05$). LY354740 alone had no effect on either measure of catalepsy. Data shown represent the mean (± SEM) of data collected from eight animals.

catalepsy is interesting because of previous studies demonstrating that nonselective mGluR agonists can induce catalepsy (Kronthaler and Schmidt, 1996). Because LY354740 is highly selective for group II mGluRs, it is likely that this mGluR-induced catalepsy is caused by activation of another mGluR subtype. Consistent with this, LY354740 produces no effect on motor activity when administered alone (Helton et al., 1998) but reduces haloperidol-induced muscle rigidity (Konieczny et al., 1998). Furthermore, agonists of group I mGluRs have physiological and behavioral effects that suggest that agonists of these receptors are likely to have catalepsy-inducing effects (Sacaan et al., 1991; Kaatz and Albin, 1995).

Other potential sites of action of group II mGluR agonists

Taken together with previous studies revealing a critical role of the STN in parkinsonian states (Guridi and Obeso, 1997; Wichmann and DeLong, 1998), the results of the present anatomical and physiological studies suggest that the behavioral effects of LY354740 are at least partially attributable to an mGluR2/3-mediated reduction in glutamate release from STN terminals. However, it is possible that actions of group II mGluR at other sites could also contribute to this effect. Although the distribution of group II mGluRs in other basal ganglia structures is somewhat limited, previous studies reveal that these receptors are present in the striatum (Testa et al., 1998) where they are involved in regulating transmission at corticostriatal synapses (Lovinger and McCool, 1995; Pisani et al., 1997). If group II mGluRs are preferentially involved in inhibiting synaptic excitation of striatal projection neurons that give rise to the indirect pathway, this could contribute to the overall behavioral effects of group II mGluR agonists. Also, it is possible that group II mGluRs present in motor regions outside of the basal ganglia, such as the cortex (Neki et al., 1996) and thalamus (Ohishi et al., 1993), could contribute to the motor effects of group II mGluR agonists.

It is interesting to note that, in addition to projecting to basal ganglia output nuclei, STN neurons also project to the dopaminergic neurons of the SNc (Kita and Kitai, 1987; Iribe et al., 1999). Furthermore, glutamate has been implicated as an excitotoxic agent in PD (Albin and Greenamyre, 1992; Rodriguez et al., 1998), suggesting that increased excitatory drive to the SNc may contribute to the progressive loss of SNc dopaminergic neurons in PD. On the basis of this, if group II mGluRs are also involved in inhibiting transmission at STN synapses in the SNc, it is possible that agonists of these receptors could reduce the component of SNc neuronal death that is mediated by STN-induced excitotoxicity. Interestingly, previous immunocytochemical studies reveal that mGluR2/3 immunoreactivity is present in the SNc (Testa et al., 1998). Furthermore, physiological studies reveal that agonists of group II mGluRs inhibit evoked EPSPs in this region (Wigmore and Lacey, 1998). Although the source of the excitatory afferents regulated by group II mGluRs in the SNc was not defined, it is possible that these EPSCs are mediated in part by activity at STN terminals. These data raise the exciting possibility that group II mGluR agonists have the potential not only for

reducing the symptoms of established PD but also for slowing the progression of PD. Future studies will be needed to define clearly the role of increased STN activity in contributing to progression of the disorder and to define rigorously the mGluR subtypes involved in regulating transmission at STN–SNc synapses.

Summary

The data presented suggest that group II mGluRs are presynaptically localized on STN terminals in the SNr and that activation of these receptors selectively reduces transmission at excitatory STN synapses in this region. Taken together with the behavioral data presented, these studies raise the exciting possibility that agonists of group II mGluRs may provide a novel, nonsurgical approach to the treatment of PD that bypasses the problems inherent with dopamine-replacement therapy. Furthermore, because group II mGluR agonists act downstream from nigrostriatal dopaminergic neurons, these compounds could be useful for the treatment of drug-induced parkinsonism in patients treated with haloperidol and other dopamine receptor antagonists that are used as antipsychotic agents. Finally, it is important to note that pallidotomy and inactivation of the STN are being explored as having therapeutic potential in other movement disorders, including dystonia and tardive dyskinesias (Vitek et al., 1998), and that increased activity in the STN is implicated in some forms of epilepsy (Deransart et al., 1996, 1998, 1999; Vercueil et al., 1998). Thus, it is conceivable that inhibition of excitatory transmission at the STN–SNr synapse with group II mGluR agonists could have broader therapeutic potential than that of L-DOPA and other drugs used for dopamine replacement in PD patients.

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Activation of Metabotropic Glutamate Receptor 5 Has Direct Excitatory Effects and Potentiates NMDA Receptor Currents in Neurons of the Subthalamic Nucleus

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The subthalamic nucleus (STN) is a key nucleus in the basal ganglia motor circuit that provides the major glutamatergic excitatory input to the basal ganglia output nuclei. The STN plays an important role in normal motor function, as well as in pathological conditions such as Parkinson's disease (PD) and related disorders. Development of a complete understanding of the roles of the STN in motor control and the pathophysiological changes in STN that underlie PD will require a detailed understanding of the mechanisms involved in regulation of excitability of STN neurons. Here, we report that activation of group I metabotropic glutamate receptors (mGluRs) induces a direct excitation of STN neurons that is characterized by depolarization, increased firing frequency, and increased burst-firing activity. In addition, activation of group I mGluRs induces a selective potentiation of NMDA-evoked currents. Immunohistochemical studies at the light and electron microscopic levels indicate that both subtypes of group I mGluRs (mGluR1a and mGluR5) are localized postsynaptically in the dendrites of STN neurons. Interestingly, pharmacological studies suggest that each of the mGluR-mediated effects is attributable to activation of mGluR5, not mGluR1, despite the presence of both subtypes in STN neurons. These results suggest that mGluR5 may play an important role in the net excitatory drive to the STN from glutamatergic afferents. Furthermore, these studies raise the exciting possibility that selective ligands for mGluR5 may provide a novel approach for the treatment of a variety of movement disorders that involve changes in STN activity.

Key words: metabotropic glutamate receptor; subthalamic nucleus; basal ganglia; Parkinson's disease; burst firing; NMDA receptor; mGluR1; mGluR5

The basal ganglia (BG) are a set of subcortical nuclei that play a critical role in motor control and are a primary site of pathology in a number of movement disorders, including Parkinson's disease (PD), Tourette's syndrome, and Huntington's disease. Recent studies reveal that a key nucleus in the BG motor circuit, the subthalamic nucleus (STN), plays an especially important role in BG function. The STN is an excitatory glutamatergic nucleus in the BG and provides the major excitatory input to the BG output nuclei, the substantia nigra pars reticulata (SNr) and the internal globus pallidus. Normal motor function requires an intricate balance between excitation of the output nuclei by glutamatergic neurons from the STN and inhibition of the output nuclei by GABAergic projections from the striatum (for review, see Wichmann and DeLong, 1997).

Interestingly, recent studies suggest that the major pathophysiological change that occurs in response to loss of nigrostriatal dopamine neurons in PD patients is an increase in activity of STN neurons. The resultant increase in synaptic excitation of GABAergic projection neurons in the output nuclei leads to a "shutdown" of thalamocortical projections and produces the motor impairment characteristic of PD (DeLong, 1990). Conversely, hyperkinetic disorders such as Huntington's disease (Reiner et al., 1988; Albin et al., 1990) and Tourette's syndrome (Albin et al., 1989; Leckman et al., 1997) are associated with decreases in STN activity. These discoveries have led to a major interest in development of novel strategies to treat these disorders by altering neuronal STN activity or STN-induced excitation of BG output nuclei. Interestingly, surgical lesions (Bergman et al., 1990; Aziz et al., 1991; Guirdi et al., 1996) and high-frequency stimulation of the STN (Benazzouz et al., 1993; Limousin et al., 1995a,b) are highly effective in treatment of PD. Development of a detailed understanding of the mechanisms involved in regulation of STN activity could lead to development of novel therapeutic agents that alter STN activity without surgical intervention.

Recent studies suggest that metabotropic glutamate receptors (mGluRs) play an important role in regulating excitability of neurons in a wide variety of brain regions, including BG structures (Conn and Pin, 1997). If mGluRs are involved in regulating excitation of STN neurons, this could provide a critical component of regulation of STN activity by glutamatergic afferents. Thus, it will be important to determine whether mGluRs are postsynaptically localized in these neurons and whether activation of mGluRs alters STN activity. To date, eight mGluR subtypes have been cloned from mammalian brain and are classified into three major groups based on sequence homologies, second messenger coupling, and pharmacological profiles (for review, see Conn and Pin, 1997). Group I mGluRs (mGluR1 and mGluR5) couple primarily to G_q, whereas group II (mGluR2 and mGluR3) and group III mGluRs (mGluRs 4, 6, 7, and 8) couple to G_i/G_o. We now report that activation of the group I mGluR mGluR5 has a dramatic excitatory effect and selectively increases NMDA receptor currents in STN neurons.

MATERIALS AND METHODS

Slice preparation for electrophysiology. Experiments were performed in STN neurons from 10- to 14-d-old Sprague Dawley rats. Rats were decapitated, the brains were removed, and a block of tissue containing the STN was isolated on ice. The tissue was mounted and immersed in an oxygenated sucrose-artificial CSF (ACSF) solution containing (in mM): 3 KCl, 1.9 $MgSO_4$, 1.2 KH_2PO_4, 2 $CaCl_2$, 187 sucrose, 20 glucose, 26 $NaHCO_3$, 0.5 pyruvate, and 0.005 glutathione, equilibrated with 95% O_2 and 5% CO_2, pH 7.4. Saggital slices (300 μm) were prepared using a manual VibroSlice (Stoelting, Chicago, IL) and then incubated at room temperature in ACSF containing (in mM): 124 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1 NaH_2PO_4, 2 $CaCl_2$, 20 glucose, 26 $NaHCO_3$, 0.5 pyruvate, and 0.005 glutathione, equilibrated with 95% O_2 and 5% CO_2, pH 7.4. In experiments requiring potassium

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channel blockade the ACSF had the following composition (in mM): 105.4 NaCl, 19.6 NaOAc, 2.5 KCl, 1.3 $MgCl_2$ 0.1 $CaCl_2$, 0.1 $CdCl_2$, 2 $BaCl_2$, 6 CsCl, 20 glucose, 26 $NaHCO_3$, 3 4-aminopyridine (4-AP), and 25 tetraethylammonium-Cl.

Electrophysiological recordings. After a 2 hr incubation, the slices were transferred to a recording chamber mounted on the stage of an Olympus Optical (Tokyo, Japan) microscope and continuously perfused at 1–2 ml/min with oxygenated ACSF containing 50 μM picrotoxin and 0.5 μM tetrodotoxin (except when firing was studied). Recordings were made with visualized patch-clamp techniques using Nomarski optics with a water immersion 40× objective. Whole-cell patch-clamp recordings were made using patch electrodes pulled from borosilicate glass on a Narishige (Tokyo, Japan) vertical puller. Electrodes were filled with (in mM): 140 potassium gluconate, 10 HEPES, 10 NaCl, 0.2 EGTA, 2 MgATP, and 0.2 NaGTP. Internal solutions used in experiments requiring potassium channel block contained 140 mM cesium methylsulfonate in place of potassium gluconate. Signals were recorded using a Warner PC-501A patch-clamp amplifier (Warner Instrument Corp., Hamden, CT) and a pClamp data acquisition and analysis system (Axon Instruments, Foster City, CA).

For measurement of NMDA and kainate-evoked currents, NMDA (100 μM–1 mM) with glycine (100 μM) or kainate (100 μM) was pressure-ejected into the slice from a low-resistance pipette using a Picospritzer (General Valve, Fairfield, NJ) at pressures ranging from 5 to 20 psi and for durations of 50–200 msec. Currents were recorded from a holding potential of −60 mV. Slices were bathed in ACSF containing 0.5 μM tetrodotoxin to block synaptic transmission. Agonists and antagonists of mGluRs were then applied by bath infusion for 5 min. NMDA receptor (NMDAR) and kainate receptor current amplitude was measured from baseline to peak of the current.

Animal perfusion and preparation of tissue for immunohistochemistry. Five male Sprague Dawley rats were deeply anesthetized with ketamine (20 mg/kg) and transcardially perfused with cold, oxygenated Ringer's solution followed by 500 ml of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (PB, 0.1 M), pH 7.4, followed by 300 ml of cold PB. The brain was removed from the skull and stored in PBS (0.01 M), pH 7.4, before being sliced on a vibrating microtome into 60 μm transverse sections. These sections were then treated with 1.0% sodium borohydride for 20 min and rinsed in PBS.

Immunohistochemistry. The sections were preincubated at room temperature in a solution containing 10% normal goat serum (NGS), 1.0% bovine serum albumin (BSA), and 0.3% Triton X-100 in PBS for 1 hr. They were then transferred to solutions containing each of four primary antibodies raised against synthetic peptides corresponding to either the C terminus of mGluR1a (Chemicon, Temecula, CA) or to residues 1116–1130 of mGluR1a (Dr. Carmelo Romano, Washington University School of Medicine, St. Louis, MO) or to the C terminus of mGluR5 (Upstate Biotechnology, Lake Placid, NY; Dr. Carmelo Romano). Antibodies were diluted at 0.5–1.0 mg/ml in a solution containing 1.0% NGS, 1.0% BSA, and 0.3% Triton X-100 in PBS. The tissue was incubated in this solution overnight at room temperature. The sections were rinsed in PBS and incubated for 1 hr at room temperature in a secondary antibody solution containing biotinylated goat-anti-rabbit IgGs (Vector Laboratories, Burlingame, CA) diluted 1:200 in the primary antibody diluent solution. After rinsing, sections were put in a solution containing 1:100 avidin–biotin–peroxidase complex (Vector). The tissue was then washed in PBS and 0.05 M Tris buffer before being transferred to a solution containing 0.01 M imidazole, 0.0005% hydrogen peroxide, and 0.025% 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) in Tris for 7–10 min. Sections were then mounted on gelatin-coated slides, dried, and coverslipped with Permount.

For immunohistochemical analysis at the electron microscopic level, the sections were treated with cryoprotectant for 20 min and transferred to a −80°C freezer for an additional 20 min. The sections were then thawed and treated with successively decreasing concentrations of cryoprotectant and finally PBS. The immunocytochemical procedure was the same as that used for studies at the light level, except that Triton X-100 was not used, and the incubation in the primary antibody was performed at 4°C for 48 hr. After DAB revelation, the sections were processed for the electron microscope. They were first washed in 0.1 M PB for 30 min and then post-fixed in 1.0% osmium tetroxide for 20 min. Afterward, they were washed in PB and dehydrated by a series of increasing concentrations of ethanol (50, 70, 90, and 100%). Uranyl acetate (1.0%) was added to the 70% ethanol to enhance contrast in the tissue. Next, the sections were exposed to propylene oxide and embedded in epoxy resin (Durcupan; Fluka, Buchs, Switzerland) for 12 hr. They were then mounted on slides, coverslipped, and heated at 60°C for 48 hr.

Five blocks (three for mGluR1a and two for mGluR5) were cut from the STN and mounted on resin carriers to allow for the collection of ultrathin sections using an ultramicrotome (Ultracut 2; Leica, Nussloch, Germany). The ultrathin sections were collected on single-slot copper grids, stained with lead citrate for 5 min to enhance contrast, and examined on a Zeiss (Thornwood, NY) EM-10C electron microscope. Electron micrographs were taken at 10,000 and 31,500× magnifications to characterize the nature of immunoreactive elements in the STN.

Drugs. All drugs were obtained from Tocris Cookson (Ballwin, MO), except that (+)-2-aminobicyclo[3.1.0]-hexane-2,6-dicarboxylate monohydrate (LY354740) was a gift from D. Schoepp and J. Monn (Eli Lilly, Indianapolis, IN), methylphenylethynylpyridine (MPEP) and 7-hydroxyiminocyclopropan-[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt) were gifts from R. Kuhn (Novartis, Basel, Switzerland), and (*S*)-(+)-2-(3′-carboxy-bicyclo[1.1.1]pentyl)-glycine (CBPG) was purchased from Alexis (San Diego, CA). All other materials were obtained from Sigma.

Data analysis. Values are expressed as mean ± SEM. Statistical significance was assessed using Student's *t* test.

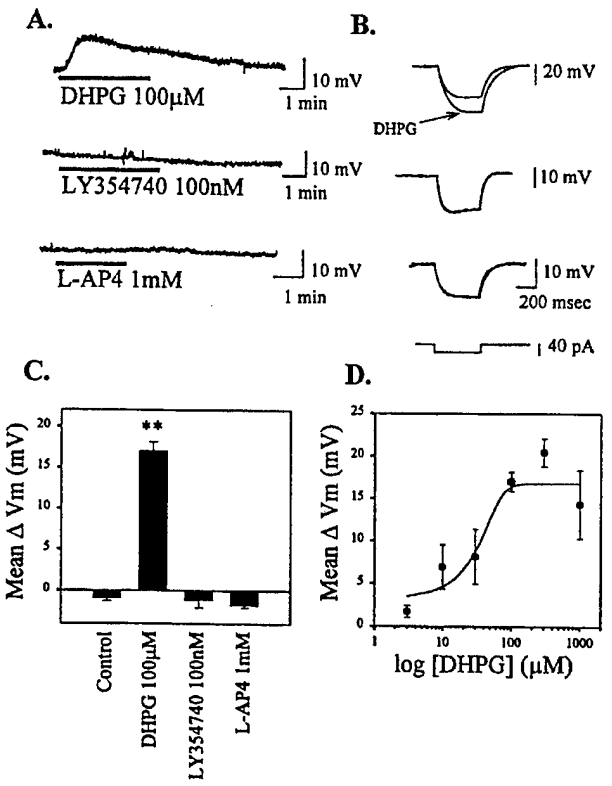

Figure 1. Group I mGluR-mediated depolarization of STN neurons. *A*, Representative current-clamp traces of membrane potential changes in response to DHPG (100 μM), LY354740 (100 nM), and L-AP-4 (1 mM) from a holding potential of −60 mV. *B*, Corresponding change in membrane input resistance accompanying the change in membrane potential. *C*, Mean data ± SEM of membrane potential changes, showing a significant depolarization by the group I-selective agonist DHPG (**$p < 0.001$). *D*, Dose–response curve of DHPG-mediated changes in membrane potential.

RESULTS

Group I mGluRs mediate depolarization of STN neurons

Previous studies suggest that STN neurons express multiple mGluR subtypes, including receptors belonging to each of the major groups of mGluRs (groups I–III) (Testa et al., 1994, 1998; Bradley et al., 1998, 1999). We took advantage of highly selective agonists of each of the mGluR groups to determine whether activation of these receptors has effects on membrane properties of STN neurons. Unless otherwise stated, all studies were performed in the presence of tetrodotoxin (TTX; 0.5 μM) to block action potential firing. The group I mGluR-selective agonist 3,5-dihydroxyphenylglycine (DHPG) (Schoepp et al., 1994) (100 μM) induced a robust depolarization of STN neurons (17.1 ± 1.1 mV; $p < 0.001$; Fig. 1*A,C*) that was accompanied by an increase in membrane input resistance (Fig. 1*B*). In voltage-clamp mode, this could be seen as an inward current with an accompanying decrease in membrane conductance (data not shown). DHPG-induced depolarization was seen in 23 of 24 cells, indicating a relatively homogeneous population of neurons. The dose–response relationship for DHPG-induced depolarization of STN neurons revealed an EC_{50} of ~30 μM (Fig. 1*D*), which is consistent with the EC_{50} value of DHPG at activation of group I mGluRs (Schoepp et al., 1994).

In contrast with DHPG, the group II selective mGluR agonist

LY354740 (100 nM) (Monn et al., 1997) had no effect on membrane potential (Fig. 1*A,C*) or input resistance (Fig. 1*B*). Likewise, the group III-selective agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP-4) (1 mM) (Conn and Pin, 1997) had no effect on the membrane potential or input resistance of STN neurons when examined in the presence of the NMDA receptor antagonists D-AP-5 (20 μM) and MK801 (10 μM) (Fig. 1*A–C*). L-AP-4 (1 mM) did induce a slight depolarization of STN neurons when applied in the absence of NMDA receptor antagonists (data not shown). This is consistent with previous reports that L-AP-4 is a weak NMDA receptor agonist (Davies and Watkins, 1982).

In other neuronal populations, activation of group I mGluRs can induce cell depolarization by inhibiting a leak potassium current (Guérineau et al., 1994) or by increasing an inward cation current (Crépel et al., 1994; Guérineau et al., 1995; Pozzo Miller et al., 1995). The finding that the DHPG-induced depolarization or inward current in STN neurons is accompanied by a decrease in membrane conductance suggests that this effect is more likely mediated by inhibition of a leak potassium current. To determine whether the DHPG-induced current has a reversal potential consistent with mediation by inhibition of a potassium current, we performed an analysis of the *I–V* relationship of the DHPG-induced current in voltage-clamp mode. *I–V* relationships were determined in the presence and absence of 100 μM DHPG by a series of voltage steps ranging from −120 to −30 mV in increments of 10 mV. *I–V* plots were determined in a total of five cells. A representative leak-subtracted *I–V* plot in the presence and absence of DHPG (100 μM) is shown in Figure 2*A*. A subtraction of the predrug *I–V* plot from that in the presence of DHPG yielded an *I–V* plot of the DHPG-induced current alone (Fig. 2*B*). DHPG induced a net outward current at hyperpolarized potentials (greater than −80 mV) and an inward current at potentials in the range of the resting potential (Fig. 2*B*). The reversal potential of the DHPG-induced current is approximately −80 mV.

A reversal potential in a hyperpolarized range coupled with the reduction in membrane conductance is consistent with the hypothesis that DHPG-induced membrane depolarization is partially attributable to inhibition of a leak potassium current. However, the reversal potential is somewhat more depolarized than the predicted equilibrium potential for potassium (−105 mV). This, coupled with the nonlinear *I–V* curve of the DHPG-induced current, suggests that other factors may also play a role in this membrane depolarization. In some other systems, group I mGluR activation can also activate inward cation currents (Crépel et al., 1994; Guérineau et al., 1995; Pozzo Miller et al., 1995). It is possible that a similar effect occurs in STN neurons. To determine whether the DHPG-induced current is solely a potassium current, we performed ion substitution experiments. Voltage-clamp experiments were performed under conditions of potassium channel block by replacement of a K^+ ion with a Cs^+ ion and inclusion of 4-AP and tetraethylammonium in the external bathing solution. DHPG-induced inward current amplitude at −60 mV was significantly reduced (10.8 ± 2.3 pA; $n = 6$) compared with that in control conditions (36.2 ± 8.1 pA; $n = 4$; $p < 0.01$; Fig. 2*E*). However, the current was not completely blocked, and the residual current is probably mediated by ions other than potassium. Consistent with this, the leak-subtracted *I–V* plot in the presence of potassium channel block has a reversal potential of −30 mV and shows that DHPG causes an inward current at potentials more negative than −30 mV and outward current at potentials more positive than −30 mV ($n = 3$; Fig. 2*C,D*).

The simplest interpretation of the data presented above is that DHPG induces depolarization of STN neurons by actions on postsynaptically localized group I mGluRs. However, it is possible that DHPG acts by inducing release of another neurotransmitter that then depolarizes STN neurons. Because all of the studies presented above were performed in the presence of TTX, it is unlikely that DHPG acts by increasing cell firing and thereby increasing neurotransmitter release. However, this does not rule out the possibility that DHPG directly depolarizes presynaptic terminals, which could lead to calcium influx and neurotransmitter release. To rule out this possibility, we performed experiments in the presence of cadmium (100 μM) in the bathing solution to block Ca^+ channel activity. Consistent with an effective block of neurotransmitter release, this concentration of cadmium completely eliminated EPSCs elicited in the STN by stimulation of the internal capsule (data not shown). However, the amplitude of the DHPG-induced current was not significantly different than that seen in control (31.1 ± 6.9 pA; $n = 4$; Fig. 2*E*).

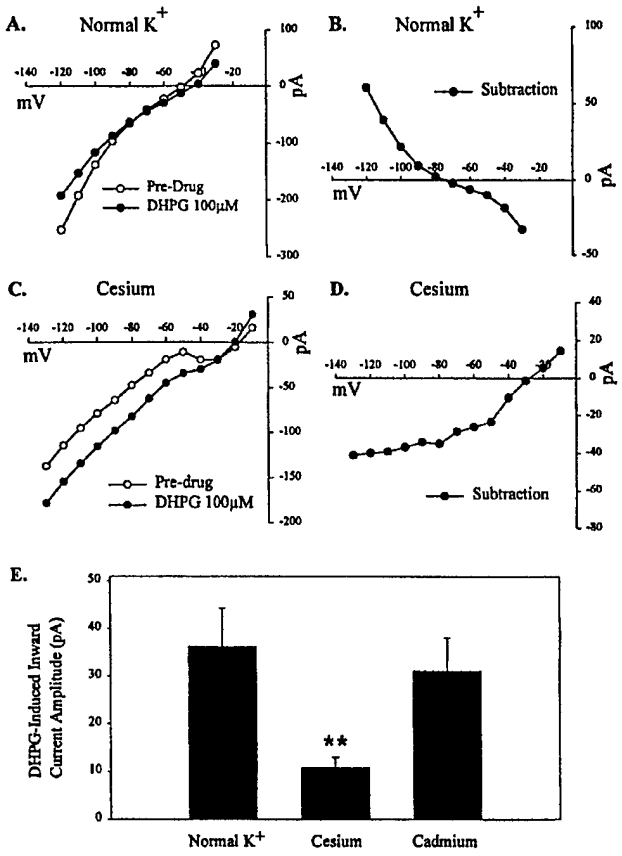

Figure 2. Ionic basis of DHPG-induced current. *A*, Leak-subtracted *I–V* plot in normal K^+ conditions in the presence and absence of DHPG (100 μM). *B*, Subtracted *I–V* plot representing DHPG-induced current alone, showing reversal potential of approximately −80 mV. *C*, Representative leak-subtracted *I–V* plot in the presence of potassium channel block and cesium. *D*, Subtracted *I–V* plot representing DHPG-induced current alone, showing reversal potential of −30 mV. *E*, Mean data ± SEM of DHPG-induced inward current amplitude (picoamperes) in voltage-clamp mode in normal potassium conditions, potassium block and cesium, and in the presence of cadmium (100 μM) (**$p < 0.01$).

When measured in the absence of TTX, the DHPG-induced depolarization was accompanied by a dramatic increase in action potential firing (Fig. 3*A*). This is consistent with a recent report that group I mGluR agonists increase extracellular single unit firing of STN neurons (Abbott et al., 1997). This increase in cell firing was completely eliminated by hyperpolarizing current injection to hold the membrane potential at the predrug level (Fig. 3*A*). This suggests that the increase in firing frequency was strictly attributable to the DHPG-induced depolarization rather than being partially mediated by other changes in membrane properties that allow the cells to fire at a higher frequency. There was no effect of DHPG on other membrane properties of the cell, including spike width, spike amplitude, and the shape or amplitude of afterhyperpolarizations (data not shown). However, DHPG did induce an increase in the incidence of burst firing, a property of STN

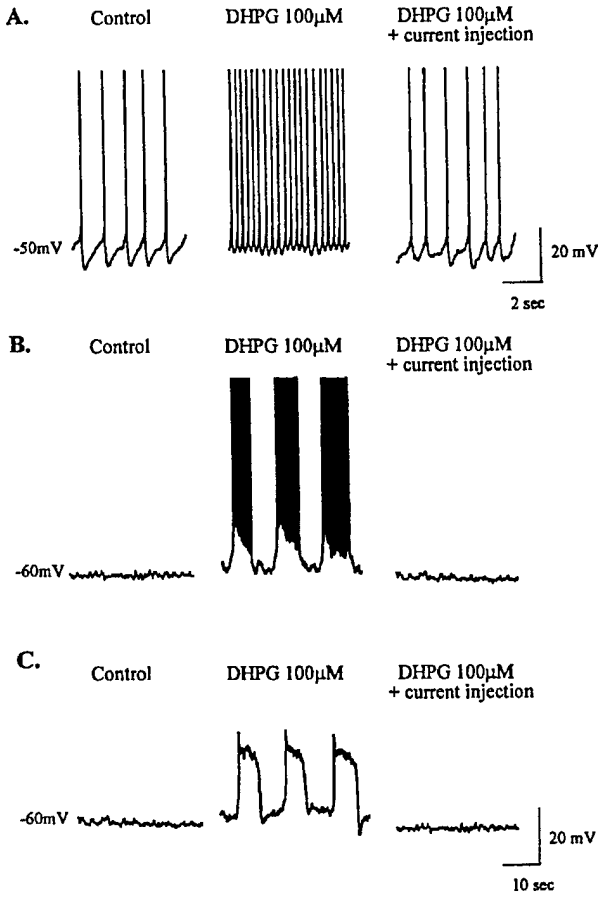

Figure 3. Postsynaptic effects of group I mGluR activation in STN neurons. *A*, Representative current-clamp traces of firing rate before drug application (at −50 mV) and dramatic increase in the presence of DHPG (100 μM) that is countered by current injection to return membrane potential to the predrug level. *B*, DHPG-mediated switch to burst-firing mode (from a holding potential of −60 mV), which is countered by hyperpolarizing current injection to maintain membrane potential at the predrug level. *C*, DHPG-mediated membrane oscillations in the presence of TTX are also countered by hyperpolarizing current injection. Action potentials are truncated in *A* and *B*. Scale bars in *C* also apply to *B*.

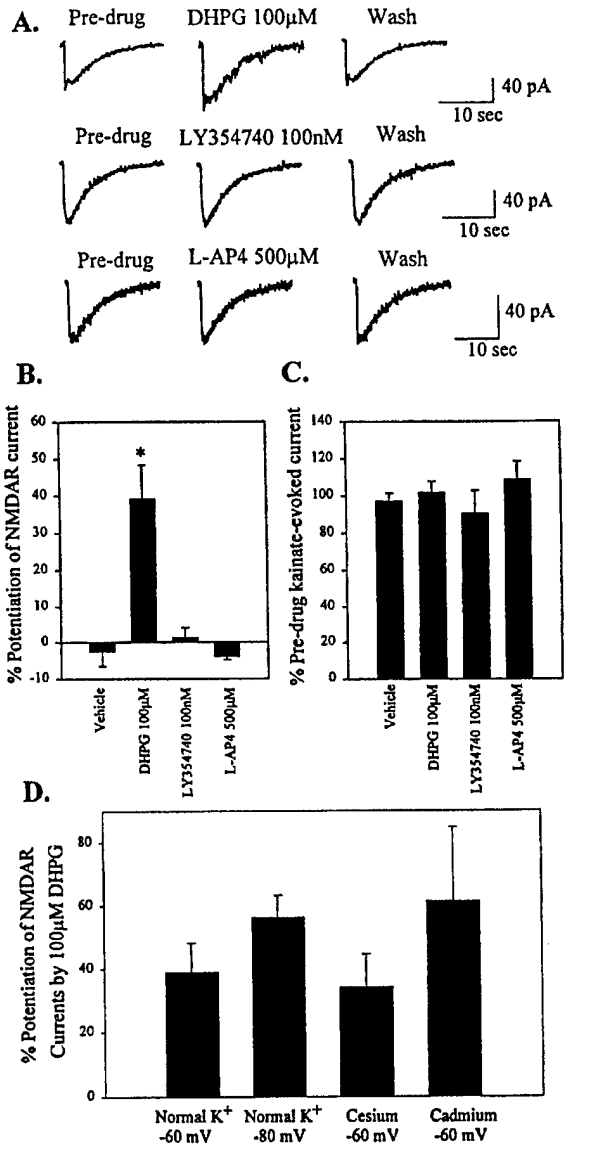

Figure 4. Activation of group I mGluRs potentiates NMDA-evoked currents in STN neurons but has no effect on kainate-evoked currents. *A*, Representative voltage-clamp traces of NMDA-evoked currents in predrug, agonist, and wash conditions. Only the group I-selective agonist caused a reversible potentiation of NMDA-evoked currents. The group II and III agonists had no effect on NMDA-evoked currents. *B*, Mean data ± SEM of percent potentiation of NMDA-evoked currents by DHPG over predrug current amplitude. DHPG caused a significant potentiation compared with vehicle (*$p < 0.05$). *C*, Mean data ± SEM of percent predrug kainate-evoked current amplitude showing no difference compared with vehicle. *D*, Mean data ± SEM of percent potentiation of NMDAR currents by DHPG in normal K^+ at −60 and −80 mV, cesium and potassium channel block at −60 mV, and in the presence of Cd^{2+} (100 μM).

neurons previously described by Beurrier et al. (1999) (Fig. 3*B*). Oscillatory activity underlying burst firing was not seen in any of 15 cells examined at a resting potential of −60 mV before DHPG treatment but was seen in 7 of 26 cells (~27%) during the DHPG-induced depolarization. In the absence of TTX, oscillatory activity was accompanied by burst firing (Fig. 3*B*). When studied in the presence of TTX, the DHPG-induced oscillatory activity underlying burst firing was seen, indicating that such activity may not be dependent on synaptic transmission and may be an intrinsic membrane property of STN neurons (Fig. 3*C*). Furthermore, burst firing was not seen in any cells treated with the group II agonist LY354740 ($n = 6$) or the group III mGluR agonist L-AP-4 ($n = 9$) when the cell membrane was held at −60 mV before drug application.

Group I mGluRs potentiate NMDA-evoked currents

The data presented above suggest that activation of group I mGluRs can exert a direct excitatory effect on STN neurons that could contribute to the overall excitatory drive to this important nucleus in the BG motor circuit. In some other brain regions, activation of mGluRs can also potentiate excitatory synaptic responses by potentiating currents through glutamate-gated cation channels. To determine the effects of mGluR agonists on the responses of STN neurons to activation of ionotropic glutamate receptors, we used pressure-evoked application of constant amounts of NMDA or kainate onto the cell. Stable baseline NMDA- or kainate-evoked currents were obtained before perfusion of the slice with mGluR agonists. Representative NMDA-evoked current traces are shown before, during, and after mGluR agonist application (Fig. 4*A*). DHPG (100 μM) caused a reversible potentiation of NMDA-evoked current amplitude (39.1 ± 9.2%; $n = 10$; $p < 0.05$; Fig. 4*B*). In contrast, the group II- and group III-selective mGluR agonists LY354740 and L-AP-4 had no effect

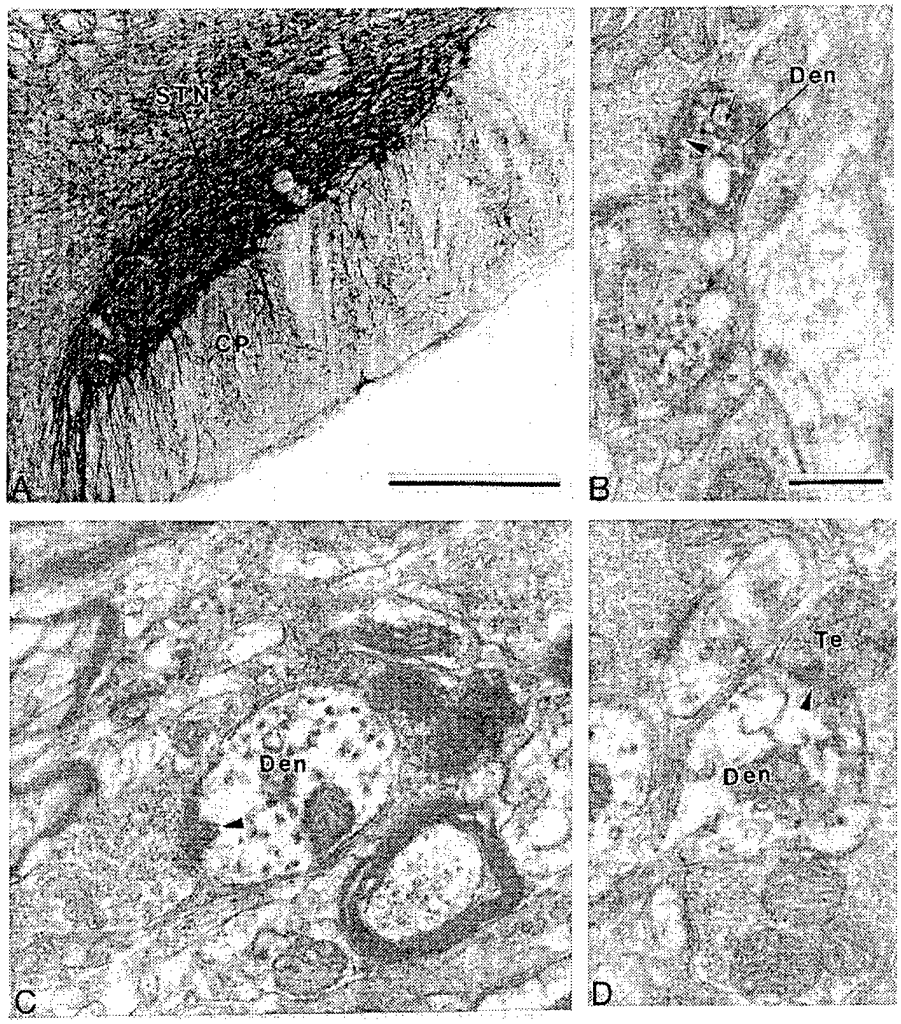

Figure 5. Immunostaining for mGluR1a in the STN. *A*, Low-power light micrograph of mGluR1a in the STN. *B, C*, High-power electron micrographs of mGluR1a-immunoreactive dendrites (*Den*) that form asymmetric synapses (*arrowheads*) with unlabeled terminals. Note that the dendrite in *B* contains vesicles (*arrows*). *D*, High-power electron micrograph of mGluR1a-immunoreactive terminal that forms an asymmetric synapse (*arrowhead*) with an immunoreactive dendrite. *CP*, Cerebral peduncle. Scale bars: *A*, 500 μm; *B–D*, 0.5 μm.

on NMDA-evoked currents (Fig. 4*A,B*). None of the group-selective mGluR agonists had any effects on kainate-evoked currents in STN neurons (Fig. 4*C*).

One concern that should be considered with studies of modulation of NMDA-evoked currents in brain slices is that it is impossible to obtain complete voltage control of the entire dendritic region of the STN neurons. Thus, it is possible that DHPG-induced potentiation of the NMDA-evoked current is attributable to depolarization of dendritic regions in which we have not achieved adequate voltage control. If so, the DHPG-induced depolarization may relieve the voltage-dependent magnesium block of the NMDA receptor and thereby cause NMDA-evoked currents to be potentiated. To test for this possibility, we performed a series of experiments to determine the effect of DHPG on NMDA-evoked currents under conditions in which the DHPG-induced depolarization is blocked. First, experiments were performed in normal K^+ concentrations when holding at the reversal potential of the DHPG-induced current (−80 mV). Also, we determined the effect of DHPG in the presence of conditions that block voltage-dependent potassium channels and thereby reduce the DHPG-induced depolarization. Under both conditions, DHPG-induced inward current was either reduced or absent. In contrast, neither manipulation significantly altered DHPG-induced potentiation of NMDA-evoked currents (Fig. 4*D*). In addition, experiments were performed in the presence of cadmium (100 μM) to ensure that the response was not caused by calcium-dependent release of another neurotransmitter from presynaptic terminals. As with the studies of DHPG-induced inward currents, cadmium had no significant effect on DHPG-induced potentiation of NMDA-evoked currents (Fig. 4*D*).

mGluR1 and mGluR5 are postsynaptically localized in STN neurons

DHPG is an agonist at both mGluR1 and mGluR5, suggesting that either of these mGluR subtypes could mediate the responses described above. We performed immunocytochemical studies with mGluR1a and mGluR5 antibodies at the electron microscopic level to determine whether both of these receptors are localized at postsynaptic sites in STN neurons.

At the light microscopic level, the STN showed strong neuropil labeling for mGluR1a and mGluR5 (Figs. 5*A*, 6*A*). In general, the immunoreactivity was found predominantly in dendritic processes, whereas the level of labeling in cell bodies was very low. In sections immunostained for mGluR1a, labeled dendrites were found in the cerebral peduncle (Fig. 5*A*), which is consistent with previous Golgi studies showing that the dendrites of neurons located along the ventral border of the STN extend ventrally into the cerebral peduncle (Iwahori, 1978; Afsharpour, 1985).

To determine the exact nature of the immunoreactive neuronal elements, we performed further analysis at the electron microscopic level. Both antibodies primarily labeled dendritic processes, which formed symmetric and asymmetric synapses with unlabeled axon terminals (Figs. 5*B–D*, 6*B–D*). In general, the labeling was seen throughout the dendrites, instead of being associated selectively with the plasma membrane (Figs. 5*B–D*, 6*B–D*). It is worth

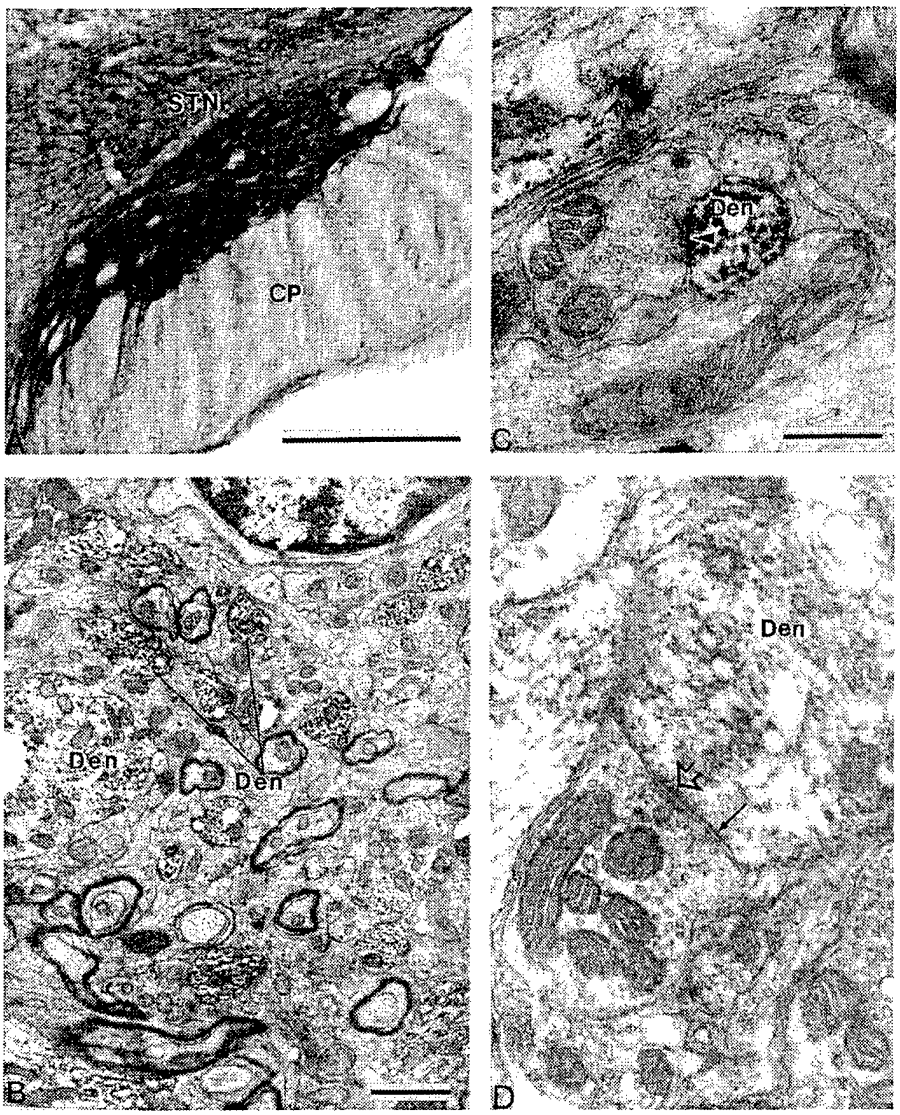

Figure 6. Immunostaining for mGluR5 in the STN. *A*, Low-power light micrograph of mGluR5 immunostaining in the STN. *B*, Low-power electron micrograph of mGluR5-immunoreactive dendrites (*Den*). *C*, High-power electron micrograph of a small mGluR5-immunoreactive dendrite that forms an asymmetric synapse (*arrowhead*) with the unlabeled terminal. *D*, High-power electron micrograph of a large mGluR5-immunoreactive dendrite that forms a symmetric synapse (*arrow*) with an unlabeled terminal. The *open arrowhead* points to a puncta adherentia. *CP*, Cerebral peduncle. Scale bars: *A*, 500 μm; *B*, 1 μm; *C*, *D*, 0.5 μm.

noting that such a pattern of labeling was also detected for mGluR5 in the SNr using both immunogold and immunoperoxidase techniques (Hubert and Smith, 1999). In addition to dendrites, mGluR1a immunoreactivity was occasionally seen in a few myelinated and unmyelinated axonal segments as well as a few axon terminals (Fig. 5*D*). Cell bodies displayed light intracytoplasmic labeling with either antibody.

Immunohistochemical studies were repeated with additional anti-mGluR1a (Romano et al., 1996) and anti-mGluR5 (Reid et al., 1995; Romano et al., 1995) antibodies and showed similar results confirming our findings of the postsynaptic localization of both of these receptor subtypes in the STN (data not shown). This is consistent with previous *in situ* hybridization studies (Testa et al., 1994) as well as studies of mGluR localization at the light level (Testa et al., 1998).

Effects of DHPG in STN neurons are mediated by mGluR5

The postsynaptic localization of both mGluR1a and mGluR5 in STN neurons suggests that either or both of these receptors could be involved in mediating the effects of DHPG. We used newly available pharmacological tools that distinguish between these two group I mGluR subtypes to further characterize the group I-mediated effects in STN. Interestingly MPEP (10 μM), a highly selective noncompetitive antagonist at mGluR5 (Bowes et al., 1999; Gasparini et al., 1999), blocked DHPG-induced membrane depolarization (4.2 ± 0.27 mV; $p < 0.001$; $n = 3$; Fig. 7*A*,*B*). In contrast, the mGluR1-selective noncompetitive antagonist CPCCOEt (100 μM) (Annoura et al., 1996; Casabona et al., 1997; Litschig et al., 1999), had no effect on DHPG-mediated depolarization of STN neurons (18.8 ± 3.0 mV; $n = 3$; Fig. 7*A*,*B*) at concentrations that have been shown to be effective at blocking mGluR1a in recombinant (Litschig et al., 1999) and native (Casabona et al., 1997) systems. These data suggest that DHPG-induced depolarization of STN neurons is mediated by mGluR5 rather than mGluR1. Consistent with this, CBPG (100 μM), a partial agonist of mGluR5 with mGluR1 antagonist activity (Mannaioni et al., 1999), mimics DHPG-induced depolarization of STN neurons. As with DHPG, the response to CBPG is blocked by MPEP but not by CPCCOEt (Fig. 7*B*).

Pharmacological analysis of mGluR-mediated potentiation of NMDA receptor currents suggests that this response is also mediated by mGluR5. As with the depolarization, MPEP blocks

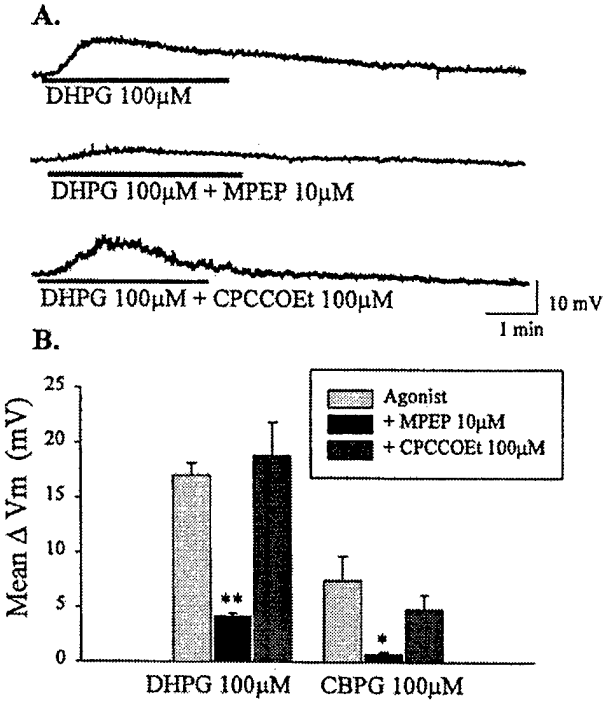

Figure 7. mGluR5 mediates group I mGluR-evoked depolarization of STN neurons. *A*, Membrane potential traces showing depolarization with DHPG (100 μM), which is blocked by the mGluR5-selective antagonist MPEP (10 μM). Membrane depolarization is not blocked by the mGluR1-selective antagonist CPCCOEt (100 μM). *B*, Mean data ± SEM of change in membrane potential showing a significant inhibition of DHPG-mediated depolarization of STN neurons by MPEP (10 μM) compared with DHPG alone (**$p < 0.001$). MPEP also significantly blocks depolarization mediated by the mGluR5-selective agonist CBPG (100 μM) (*$p < 0.05$).

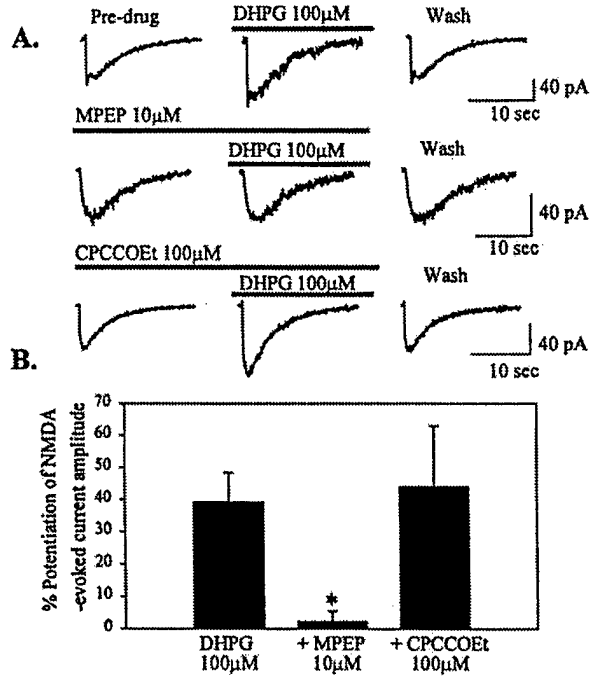

Figure 8. mGluR5 mediates group I mGluR-induced potentiation of NMDA-evoked currents. *A*, Current traces of NMDA-evoked currents before, during, and after application of DHPG (100 μM). The potentiation is blocked by MPEP (10 μM) but not CPCCOEt (100 μM). *B*, Mean data ± SEM of percent potentiation of NMDA-evoked currents by DHPG over predrug conditions. MPEP (10 μM) significantly blocks potentiation of NMDA-evoked current compared with DHPG alone (*$p < 0.05$).

DHPG-induced potentiation of NMDA-evoked currents (2.3 ± 3.2%; $p < 0.05$; $n = 5$), whereas CPCCOEt is without effect on this potentiation (44.2 ± 18.9%; $n = 6$; Fig. 8*A,B*).

DISCUSSION

The data presented reveal that group I mGluRs are postsynaptically localized on neurons in the STN and that activation of these receptors leads to a direct depolarization of STN neurons. In most cells, the DHPG-induced depolarization was accompanied by an increase in firing frequency with no obvious effects on the recently characterized stable oscillations of STN neuronal firing (Bevan and Wilson, 1999). However, in approximately one-third of the cells examined, DHPG induced a switch in the firing pattern from the characteristic single-spike firing mode to a burst-firing mode that was recently characterized in detail by Beurrier et al. (1999). In addition, DHPG induced a selective increase in NMDA receptor currents in STN neurons. These combined effects of group I mGluR activation could provide an important component of the net excitatory drive elicited by activity of the major excitatory afferents to the STN from the cortex or thalamus. However, it is important to note that activation of group I mGluRs has also been shown to induce presynaptic effects on glutamate release in some brain regions (Gereau and Conn, 1995; Manzoni and Bockaert, 1995; Rodriguez-Moreno et al., 1998). If group I mGluRs have similar effects in the STN, the net effect of group I mGluR activation in this region will ultimately depend on a combination of presynaptic and postsynaptic effects.

Immunohistochemistry studies revealed that both mGluR1a and mGluR5 are postsynaptically localized in STN neurons. Interestingly, pharmacological analysis suggested that each of the responses studied was mediated by mGluR5, with little or no contribution of mGluR1. This finding suggests that although STN neurons contain both group I mGluR subtypes, there is a segregation of function of these two receptors. It is possible that mGluR1 also plays important roles in regulating STN functions that were not measured in the present studies. Future studies of the roles of mGluR1 in these cells may shed important light on the functions of expression of multiple subtypes of closely related receptors by a single neuronal population.

Implications of mGluR5 actions for treatment of PD

One of the most interesting implications of the finding that mGluR5 is involved in regulating activity and NMDA receptor currents in STN neurons is the possibility that antagonists of this receptor could provide novel therapeutic agents that could be useful for treatment of PD. Traditional dopamine replacement strategies for PD treatment tend to lose efficacy over time, and patients begin to experience serious adverse effects, including motor fluctuations (Poewe and Granata, 1997). Because of this, a great deal of effort has been focused on developing a detailed understanding of the circuitry and function of the BG in the hopes of developing novel therapeutic approaches for the treatment of PD. Interestingly, a large number of animal and clinical studies reveal that loss of nigrostriatal dopamine neurons results in an increase in activity of the STN and that an increase in STN-induced excitation of the output nuclei is ultimately responsible for the motor symptoms of PD (for review, see DeLong, 1990; Wichmann and DeLong, 1997). These findings suggest that pharmacological agents that reduce the excitatory drive to the STN or otherwise reduce STN activity could provide a therapeutic effect in PD patients.

The data reported here suggest that mGluR5 may be a particularly interesting candidate as a receptor that could regulate STN output. Of particular interest is the finding that mGluR5 activation increases burst firing of STN neurons. For instance, previous studies suggest that a transition of STN neurons from single-spike

activity to a burst-firing mode is one of the characteristics of parkinsonian states in rats and nonhuman primates (Hollerman and Grace, 1992; Bergman et al., 1994; Hassani et al., 1996) as well as parkinsonian patients (Benazzouz et al., 1996; Rodriguez et al., 1997). The finding that membrane oscillations underlying burst firing occur in the presence of TTX is consistent with the findings of Beurrier et al. (1999) and suggests that burst firing is, in part, an intrinsic property of STN neurons. However, these data do not rule out the possibility that synaptic mechanisms also participate in induction of burst firing (Plenz and Kitai, 1999). Consistent with the hypothesis that group I mGluRs can increase the output of STN *in vivo*, Kaatz and Albin (1995) recently reported that injection of group I mGluR agonists into the STN induces rotational behavior. Furthermore, Kronthaler and Schmidt reported that (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1996) and (2*S*,3*S*,4*S*)-α-carboxycyclopropyl glycine (1998), agonists of both group I and group II mGluRs, induce catalepsy in rats. These effects are not mimicked by a highly selective agonist of group II mGluRs (Marino et al., 1999b; Bradley et al., 2000), suggesting that it is likely mediated by mGluRs belonging to group I. Taken together, these findings raise the exciting possibility that mGluR5 antagonists could reduce STN activity and thereby provide a therapeutic benefit to PD patients.

For antagonists of group I mGluRs to be effective in the treatment of PD, mGluR5 must be physiologically activated by endogenous glutamate release onto STN neurons from various glutamatergic afferents. We made several attempts to elicit slow mGluR-mediated synaptic responses using single-pulse stimuli as well as stimulus trains of varying frequencies and durations. Unfortunately, we were unable to reliably elicit mGluR-mediated EPSPs or EPSCs in our slices (our unpublished findings). Although disappointing, this is not surprising, because mGluR-mediated slow EPSPs have also been difficult to measure in other brain regions, except those in which there is a laminar or other organization in which glutamatergic afferent pathways are not severed by slice preparation. In addition to severing afferent projections during slice preparation, mGluR-mediated slow EPSPs are often difficult to measure because of the small size of the events as well as the distance between the recording site in the soma and the distal dendrites where the slow EPSP is likely generated. Because of this, failure to measure a slow EPSP does not imply that mGluRs are not synaptically activated *in vivo*. Also, we often elicited slow EPSPs that were not blocked by antagonists of known receptors and could have occluded an mGluR-mediated slow EPSP (our unpublished findings). Ultimately, the question of whether mGluR5 in the STN is activated by endogenous glutamate may require *in vivo* electrophysiological and/or behavioral studies in which mGluR5 antagonists are injected into this structure.

Potential therapeutic effects of mGluR5 agonist actions in the STN

In addition to the potential utility of mGluR5 antagonists in treatment of PD, it is important to point out that the actions of mGluR5 agonists could provide a therapeutic benefit in some other motor disorders, such as Tourette's syndrome and Huntington's disease. Tourette's syndrome is a relatively common neuropsychiatric disorder that is characterized by motor and phonic tics that can include sudden repetitive movements, gestures, or utterances. According to current models, Tourette's syndrome is associated with an increase in striatal dopamine or in the dopamine sensitivity of striatal neurons that has effects that are opposite of those seen in PD patients (Albin et al., 1989; Leckman et al., 1997). Huntington's disease is another hyperkinetic disorder that is thought to be caused by a selective loss of striatal spiny neurons that gives rise to the indirect pathway and, consequently, a decrease in STN activity (Reiner et al., 1988; Albin et al., 1990). On the basis of this, it is possible that selective mGluR5 agonists could provide a therapeutic benefit to patients suffering from these hyperkinetic disorders by increasing activity of STN neurons.

Roles of group I mGluRs in other basal ganglia nuclei

Interestingly, agonists of group I mGluRs have actions in other areas of the basal ganglia motor circuit that could complement their actions in the STN. For instance, group I mGluRs, and especially mGluR5, are heavily localized in the striatum (Shigemoto et al., 1993; Tallaksen-Greene et al., 1998), where agonists of these receptors induce excitatory effects similar to those described here in the STN (Calabresi et al., 1993; Colwell and Levine, 1994; Pisani et al., 1997). Furthermore, recent behavioral studies reveal that injection of group I mGluR agonists into the striatum induces turning behavior that is accompanied by an increase in activity of neurons in the STN and BG output nuclei (Sacaan et al., 1991, 1992; Kaatz and Albin, 1995; Kearney et al., 1997). Group I mGluRs are also present in the SNr (Hubert and Smith, 1999). Recent physiological studies from our laboratory suggest that activation of these receptors has direct excitatory effects and decreases evoked IPSCs (Marino et al., 1999a) in SNr neurons. Taken together, these data suggest that group I mGluRs function at multiple levels of the BG circuit to lead to a net increase in activity of neurons in the output nuclei. Thus, in addition to the STN, both antagonists and agonists of group I mGluRs could act at the levels of the striatum and SNr to provide a therapeutic benefit in the treatment of PD or hyperkinetic disorders, respectively.

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